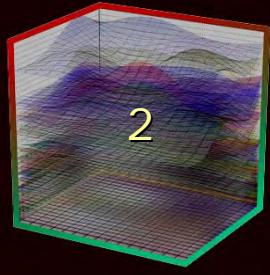


Prediction system for Chemical Safety Using PERCELLOME TOXICOGENOMICS

Kanno Jun, Aisaki Ken-ichi, Igarashi Katsuhide, Nakatsu
Noriyuki, Kitajima Satoshi, Kodama Yukio

Division of Cellular and Molecular Toxicology,
Biological Safety Research Center,
National Institute of Health Sciences,
Tokyo, Japan



Percellome Projects

Aim:

Comprehensive Gene Cascade Database by
Phenotype-Independent Approach

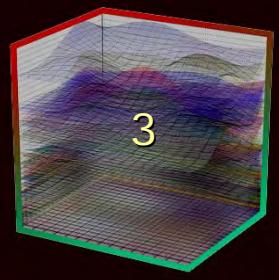
(we cannot do phenotypic anchoring for all genes).

Ultimate Goal:

Virtual mouse, virtual human *in silico*

Tentative Goal:

High-Resolution, Mechanism-based Toxicology to reinforce Traditional Toxicology
(faster, cheaper, more accurate)

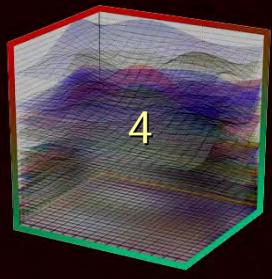


Mechanism-based modernization



Development of Cascade Database by All genes

Not all cascade accompanies phenotype



Analogy....

Electron Microscopy and Light Microscopy

Needed to write a new text book

Needed to accumulate data

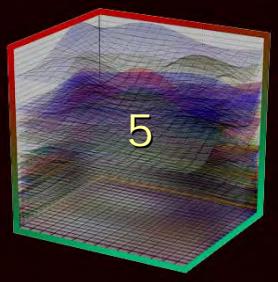
..... it took 10~20 years to write text books

Toxicogenomics and Traditional Toxicology

Need to write a new text book for practice

Need to accumulate data

..... hopefully within 10 years !?



Percellome Method

Obtain transcriptome data in
copy number of mRNA per one cell (average)
in order to
compare the accumulated transcriptome data.

MHLW* Toxicogenomics Projects

*Ministry of Health Labour and Welfare

- Toxicogenomics Project (2002~2006, 5y)

NIHS +17 Pharmaceuticals

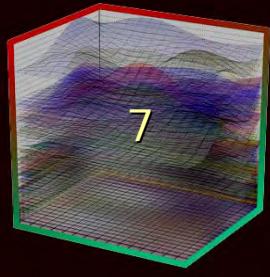
- rat (oral; liver, kidney) 150 chem, single/ repeat exposure
- + In vitro (rat primary hepatocyte, human primary hepatocyte)
- Now @ Nat'l Institute of Biomedical Innovation (Osaka) 2005~2006
- 2nd round project at Osaka (2007~2011)

- PerceLlome Project (Mouse)

Chemical Safety Database

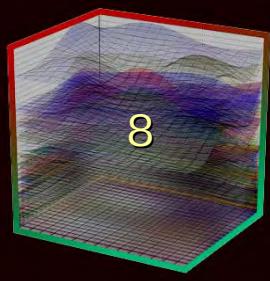
- Div. Cellular and Molecular Toxicology/ BSRC/ NIHS

- TTG1: 2003~2005 (single exposure), oral, liver, kidney 90 chem
- ITG: 2005~2007 Inhalation Toxicogenomics
- TTG2: 2006~2008 (repeated exposure, multi-organ etc.), oral
- FTG: 2004~ Fetus (developmental)
- NTG: 2006~ Behavior -> Brain TG (HC, BS, Cx, Cl)
- (Food TG): 2007~ Functional Health Food (CoQ10, α -lipo)



Why mouse?!

- Gene Knockout Organism data will bring us Objective data, and therefore important for writing a “toxicogenomics text book” (validation)
 - p53 KO
 - ER α/β KO, KI
 - hSXR KI
 - etc.



Methodology article

Open Access

"Per cell" normalization method for mRNA measurement by quantitative PCR and microarrays

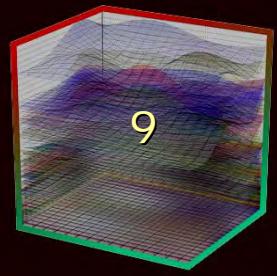
Jun Kanno *†¹, Ken-ichi Aisaki^{†1}, Katsuhide Igarashi¹, Noriyuki Nakatsu¹, Atsushi Ono¹, Yukio Kodama¹ and Taku Nagao²

Address: ¹Division of Cellular and Molecular Toxicology, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan and ²President, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

Email: Jun Kanno* - kanno@nihs.go.jp; Ken-ichi Aisaki - aisaki@nihs.go.jp; Katsuhide Igarashi - igarashi@nihs.go.jp; Noriyuki Nakatsu - n-nakatsu@nihs.go.jp; Atsushi Ono - Atsushi@nibio.go.jp; Yukio Kodama - kodama@nihs.go.jp; Taku Nagao - nagao@nihs.go.jp

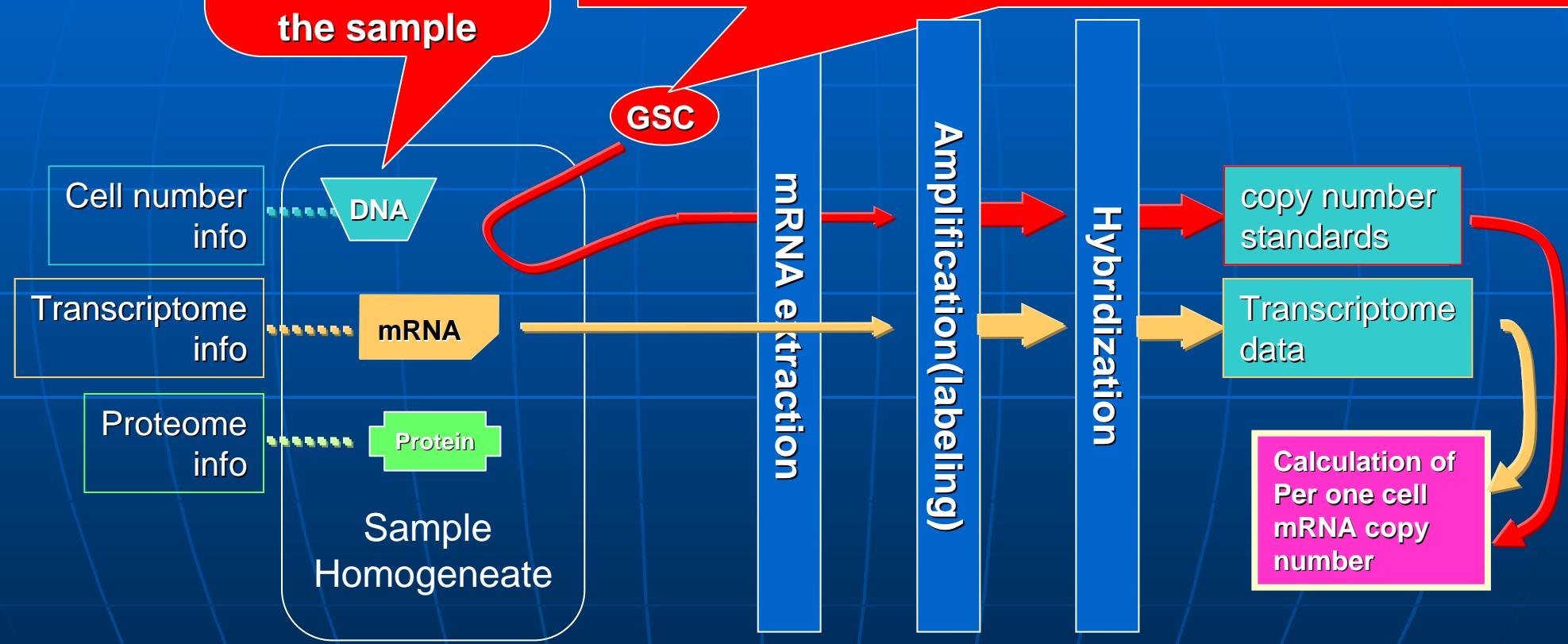
* Corresponding author †Equal contributors

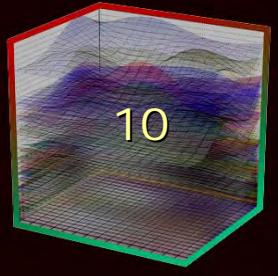
Open Access
on line journal: **BMC Genomics.** 2006 Mar 29;7(1):64
PMID: 16571132



Measure the DNA quantity and calculate the cell number of the sample

**Spike-in the mRNAs that do not cross-hybridize with sample mRNA
(Grade-dosed Spike Cocktail) in proportion to the sample cell number. This cocktail contains 5 mRNAs of known copy numbers will serve as standards of mRNA copy number.**





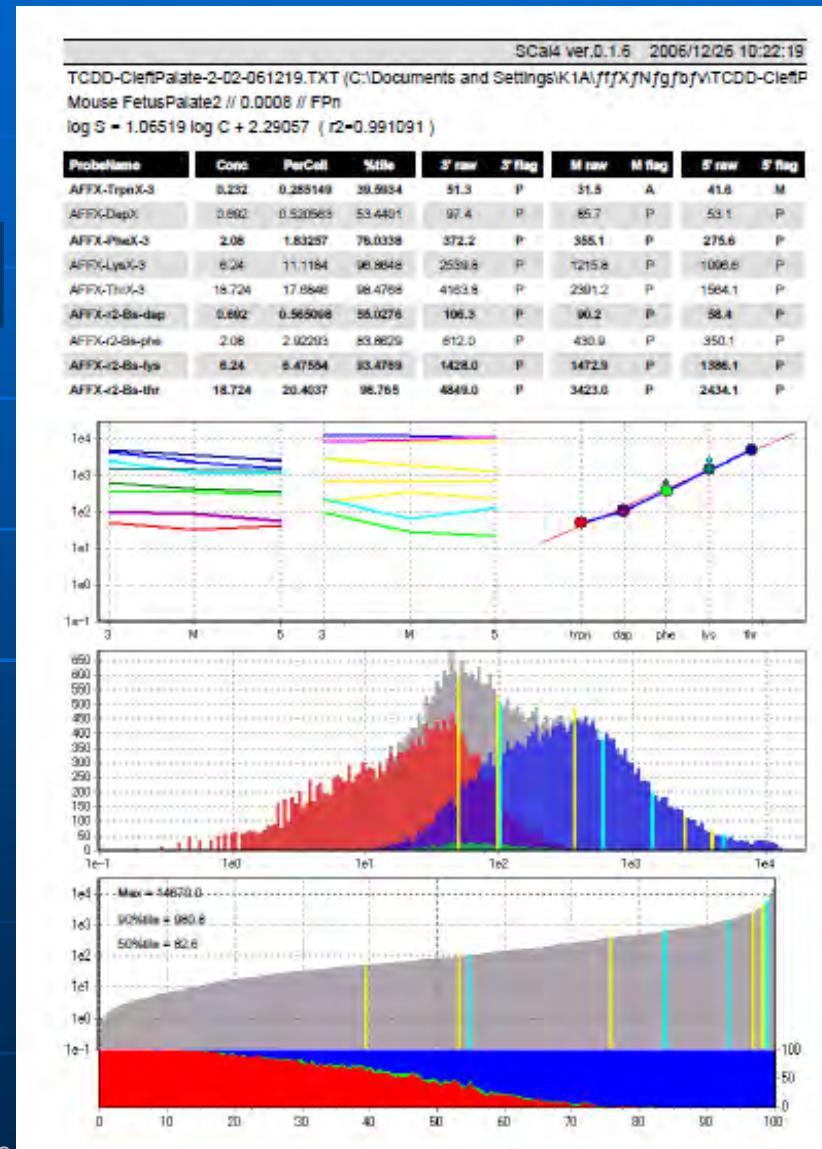
Percellome Normalization, calculation and QC (for microarray)

SCal 4

Affymetrix GeneChip
(Mouse 430 v2
Rat, Human, Xenopus)

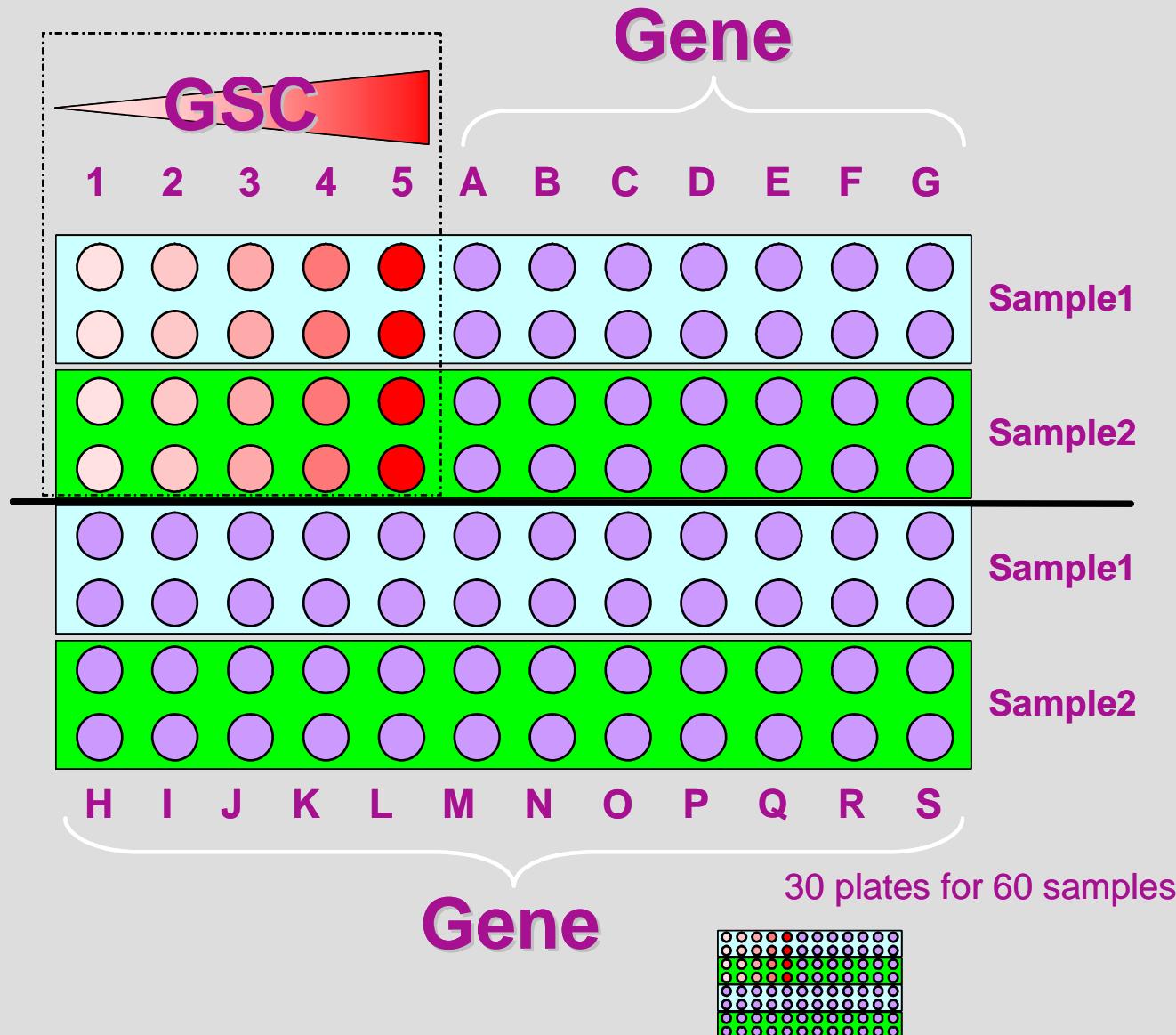
Exon Array
(human and mouse)
now under trial

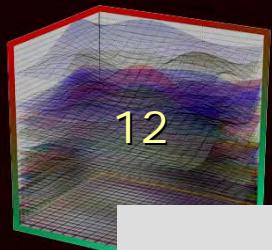
2007-05-21~23 EPA NCCT ISF @ RTP, NC



Percellome Quantitative-PCR

(by ABI PRISM 7900HT / SYBR Green)

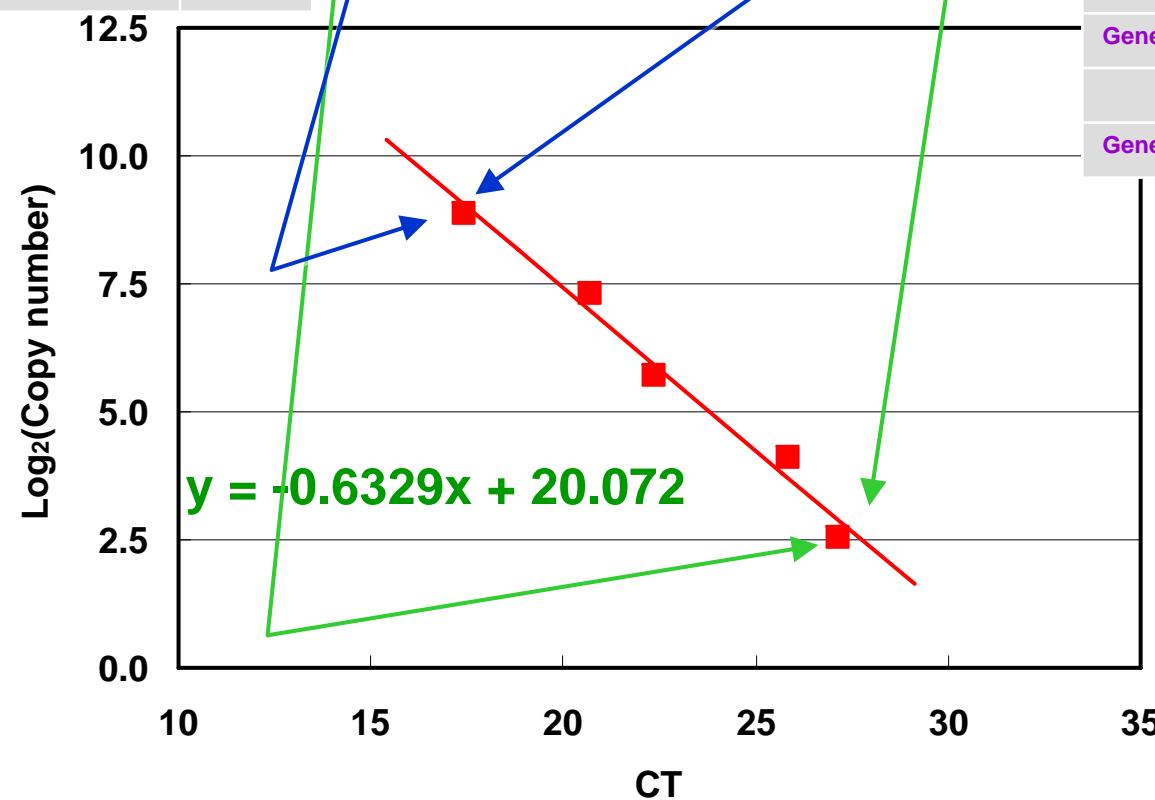


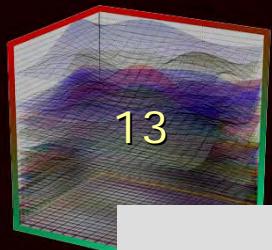


Perclome Q-PCR normalization

Copy number / cell	
GSC1:AFFX-TrpnX-3_at	5.8
GSC2:AFFX-DapX-3_at	17.4
GSC3:AFFX-PheX-3_at	52.2
GSC4:AFFX-LysX-3_at	156.6
GSC5:AFFX-ThrX-3_at	469.8
GeneA	???

CT	
GSC1:AFFX-TrpnX-3_at	27.13
GSC2:AFFX-DapX-3_at	25.85
GSC3:AFFX-PheX-3_at	22.38
GSC4:AFFX-LysX-3_at	20.72
GSC5:AFFX-ThrX-3_at	17.41
GeneA	22.92
GeneB	22.34
GeneC	24.47
GeneX	28.44
GeneS	22.30

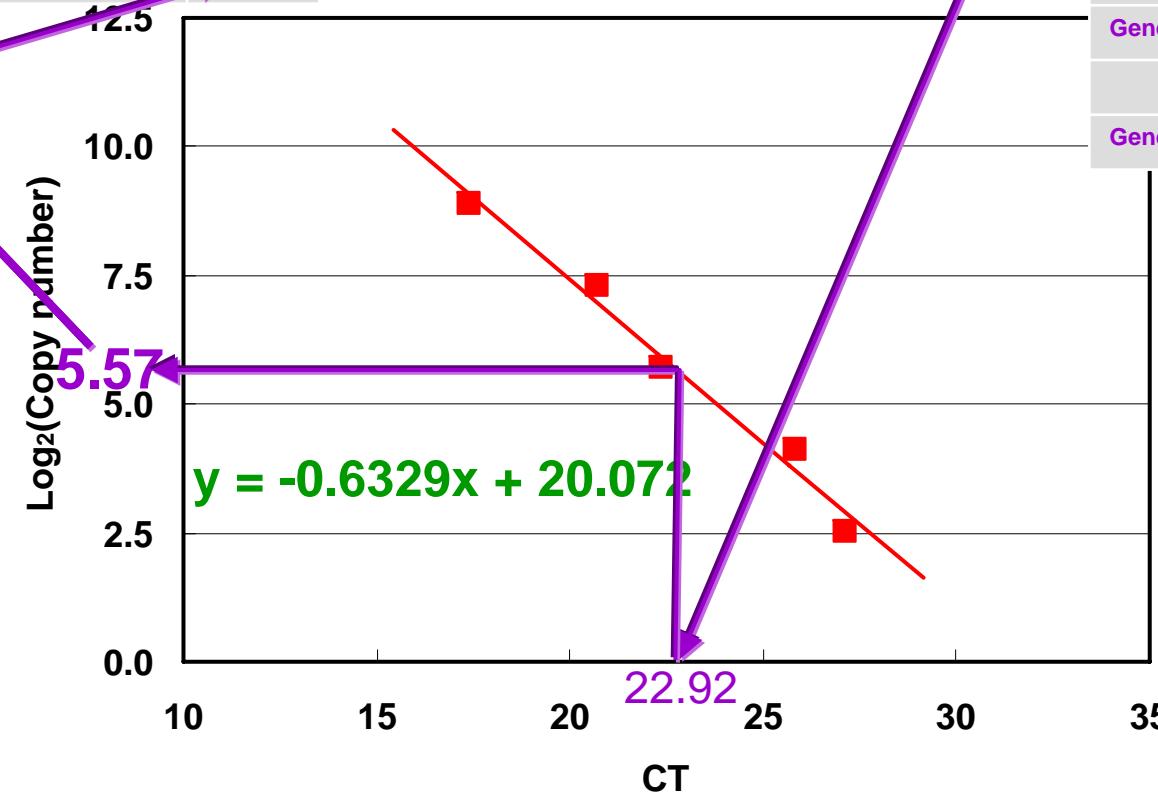




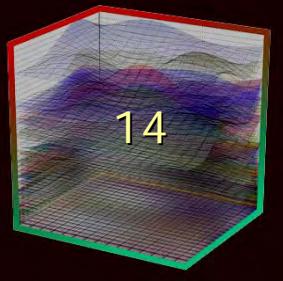
Percellome Q-PCR normalization

Copy number / cell	
GSC1:AFFX-TrpnX-3_at	5.8
GSC2:AFFX-DapX-3_at	17.4
GSC3:AFFX-PheX-3_at	52.2
GSC4:AFFX-LysX-3_at	156.6
GSC5:AFFX-ThrX-3_at	469.8
GeneA	

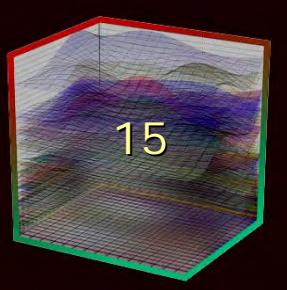
$$2^{5.57} = 47.5$$



CT	
GSC1:AFFX-TrpnX-3_at	27.13
GSC2:AFFX-DapX-3_at	25.85
GSC3:AFFX-PheX-3_at	22.38
GSC4:AFFX-LysX-3_at	20.72
GSC5:AFFX-ThrX-3_at	17.41
GeneA	22.92
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GeneC	24.47
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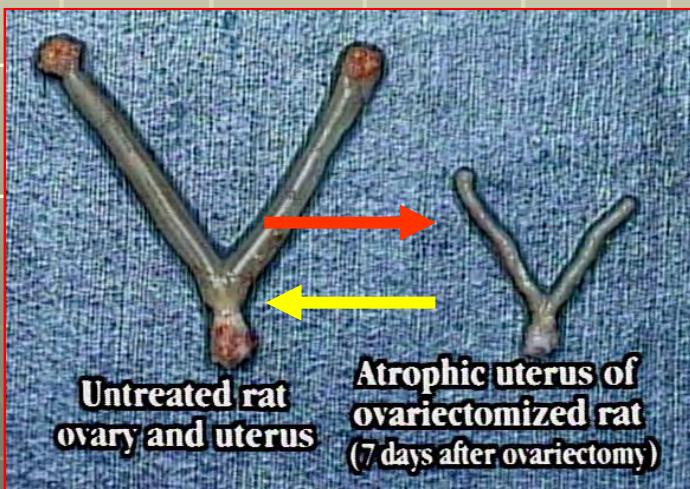


Some Features of Percellome Method

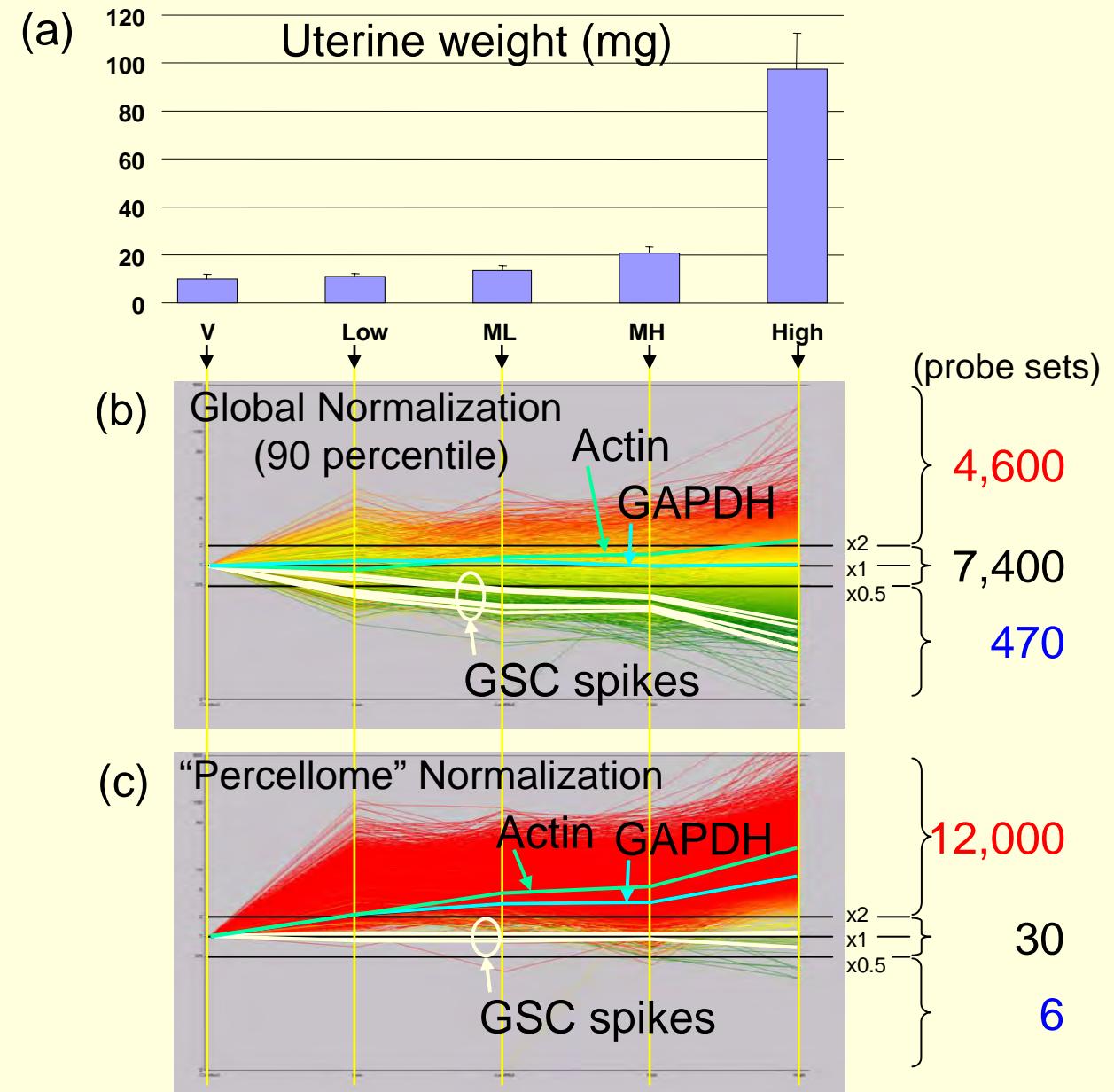


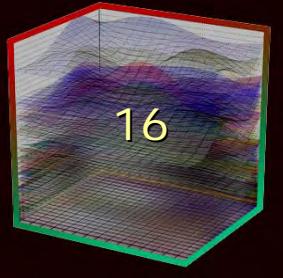
Profile-independent normalization

Ovariectomy



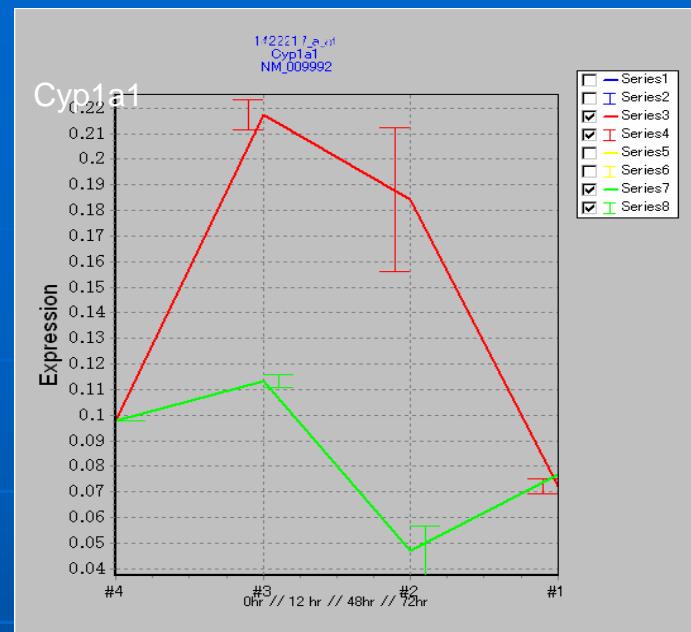
Chemical (estrogenic)
effect



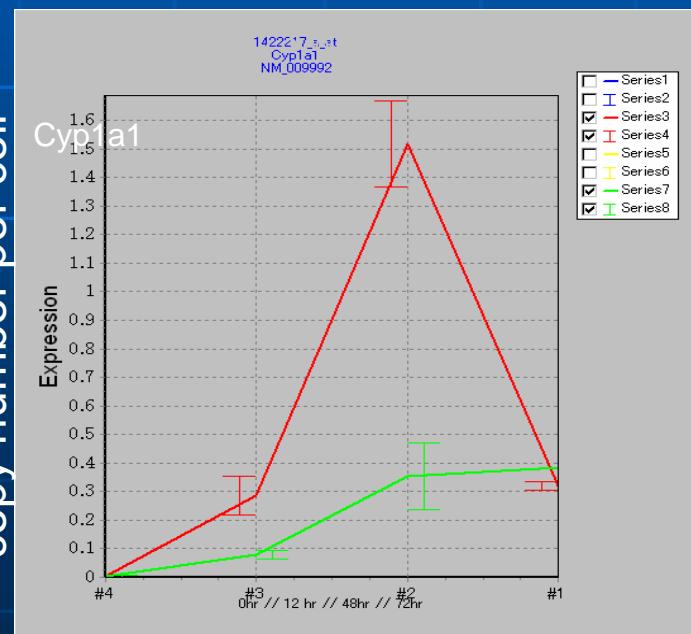


In vitro Experiment

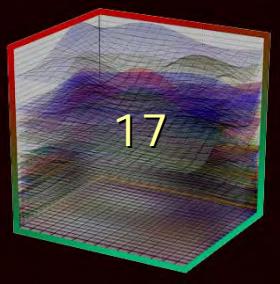
Induction of Cyp1a1 in cultured cell treated by an AhR ligand, time course study



Global Normalization



Percellome



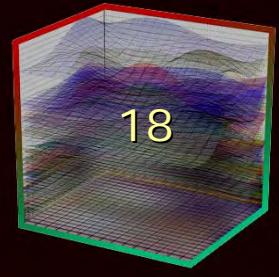
Percellome Toxicogenomics Database

Single oral gavage:

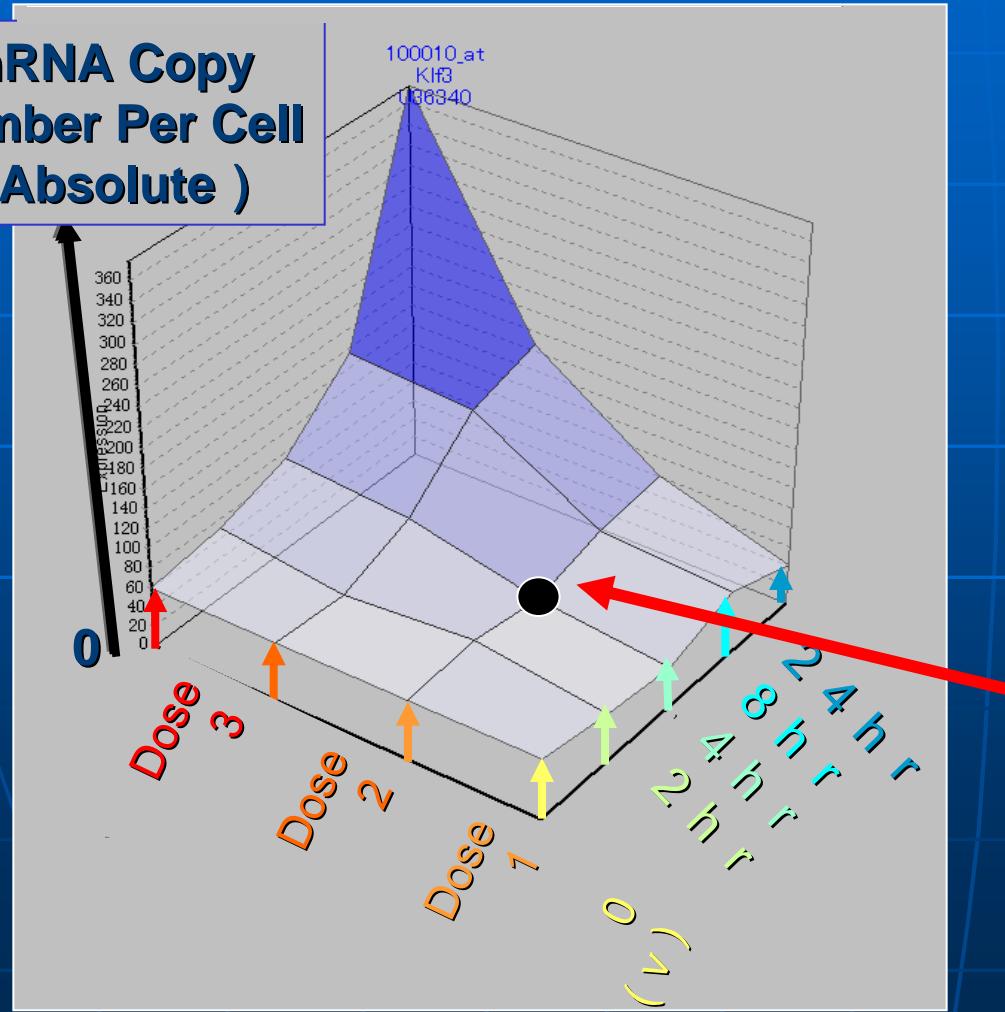
Dose selection:

No morphologic change in 24 hrs

No overt symptoms in 24 hrs

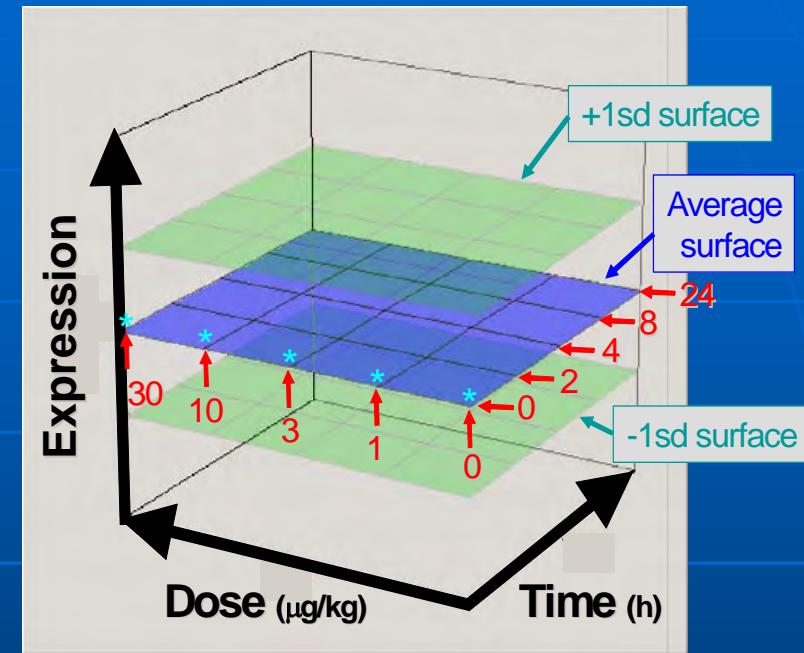


**mRNA Copy
Number Per Cell
(Absolute)**



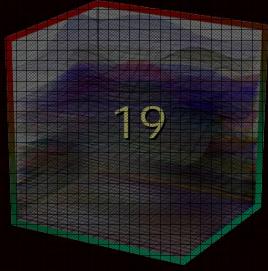
2007-05-21~23 EPA NCCT ISF @ RTP, NC

Millefeuille data (MF surface data)



One point:
Triplicate
(three GeneChips)
Mean \pm SD
 $4 \times 4 \times 3 = 48$ animals

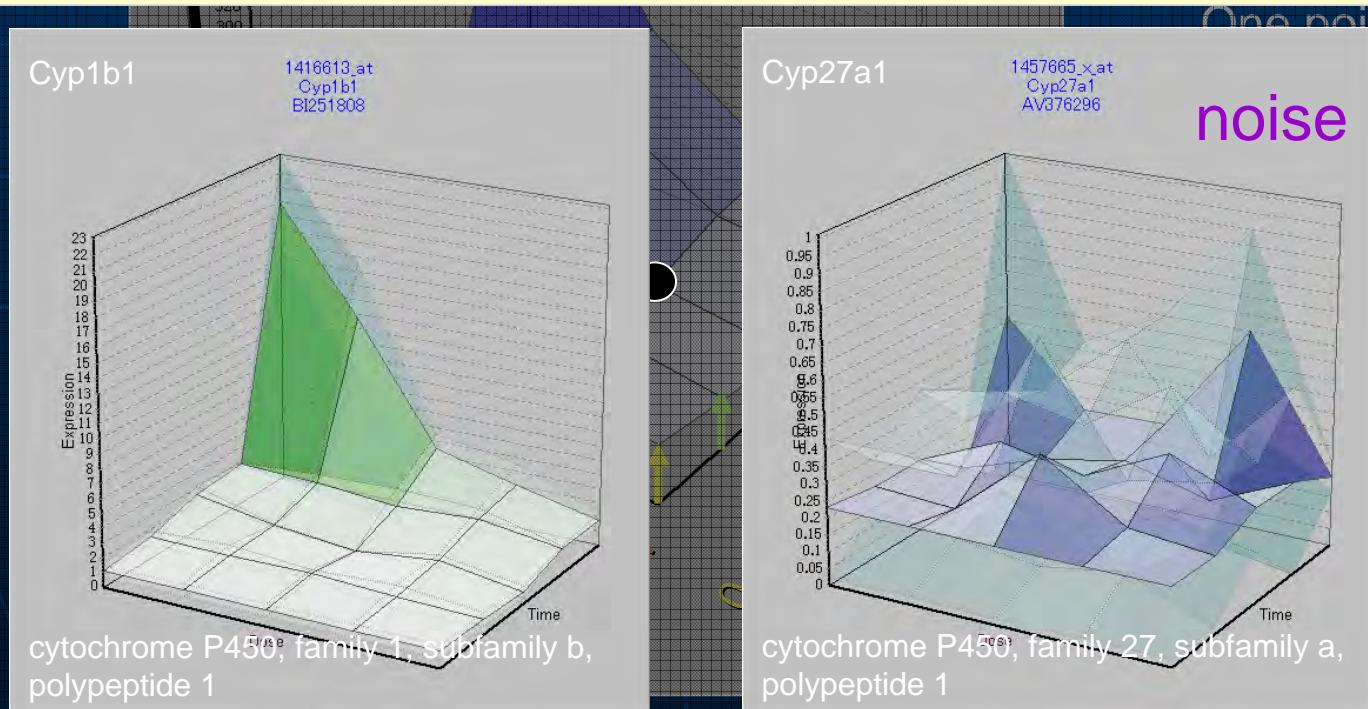


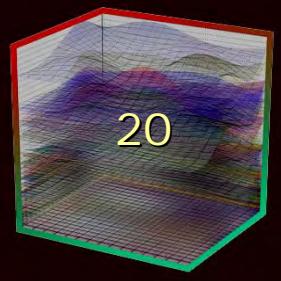


Millefeuille data (MF surface data)

mRNA Copy 100010_at

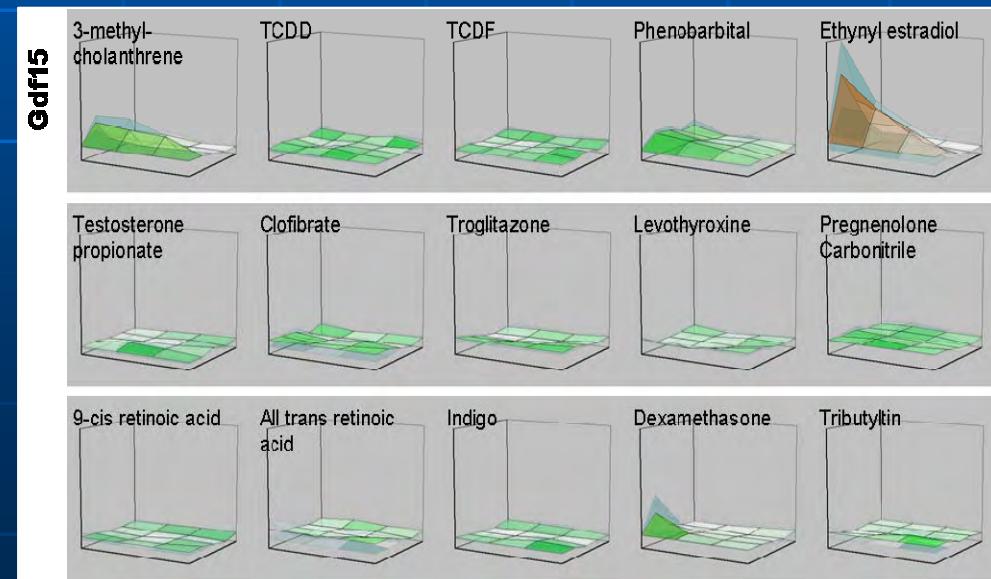
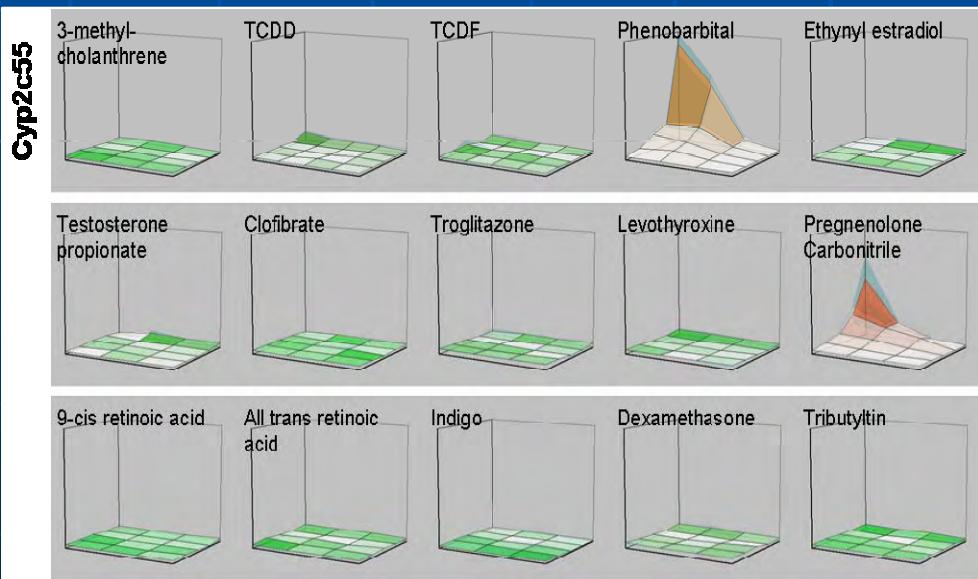
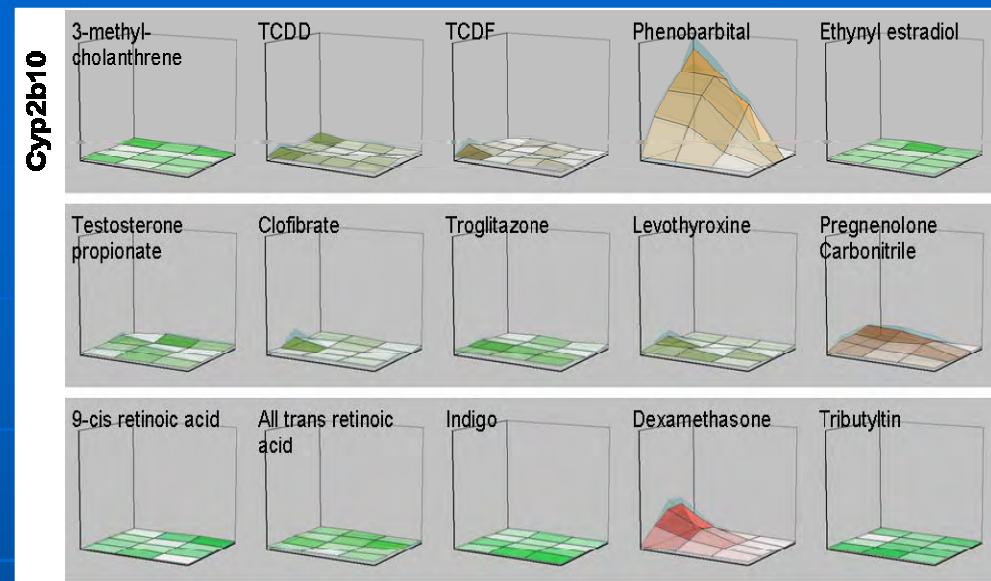
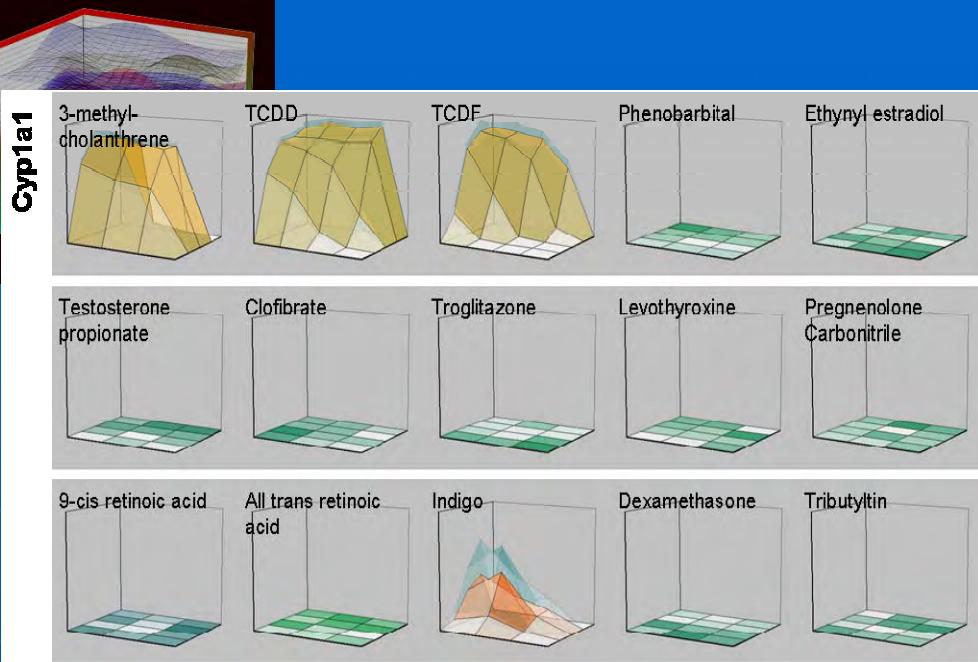
Biologist-friendly!
Easy to check the data by eyes

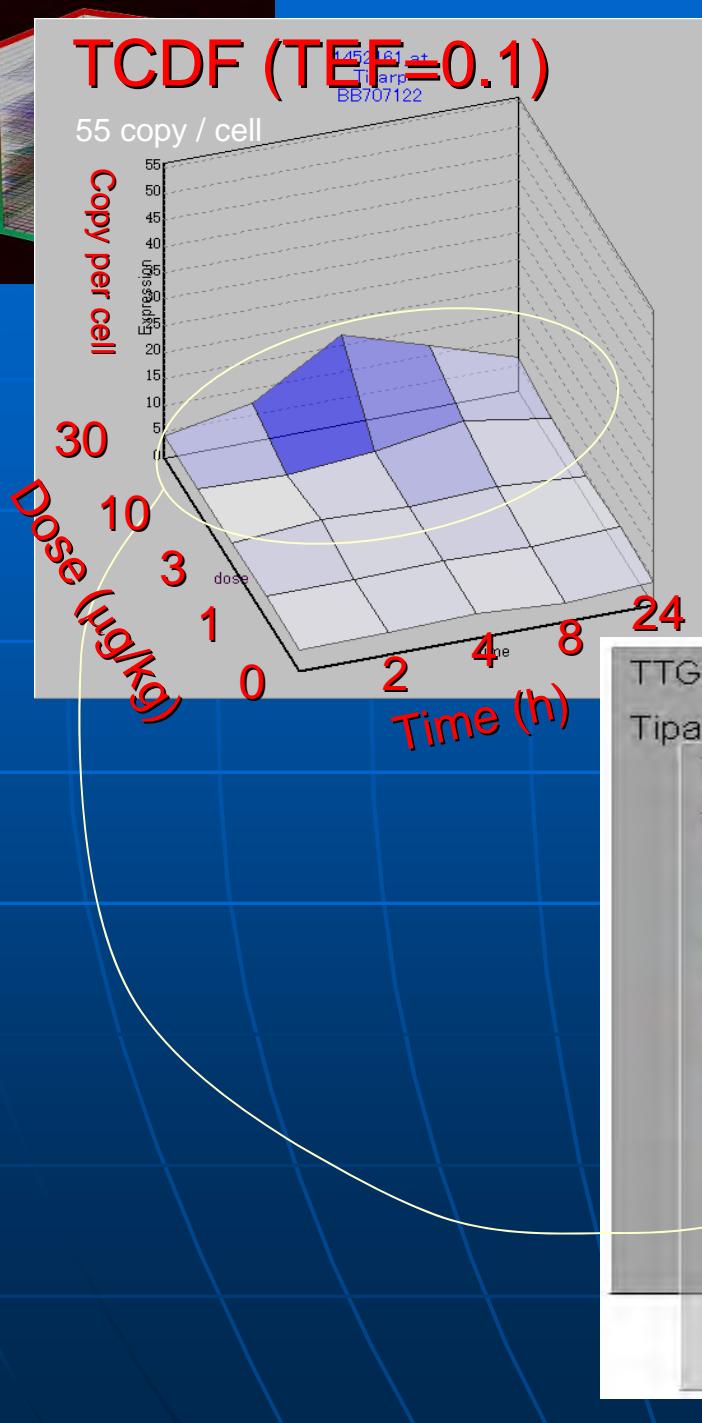




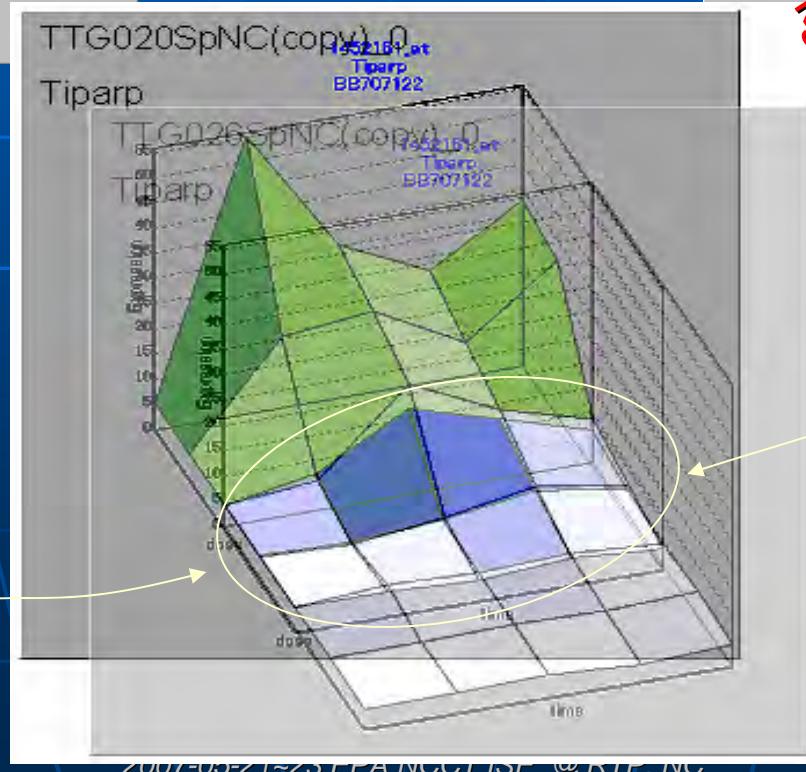
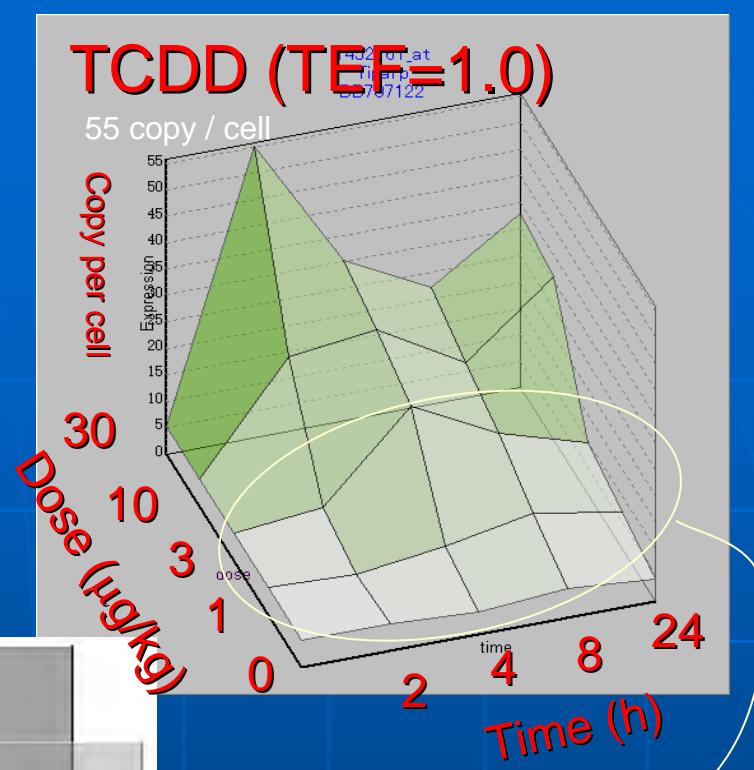
Data base Chemicals

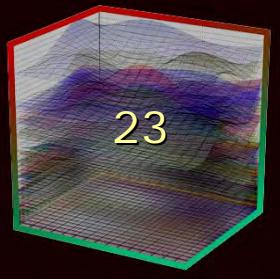
Medicine	Isoniazid Cisplatin (Transplatin) Acetaminophen Aspirin Ibuprofen Dexamethasone Omeprazole Phenobarbital Valproic Acid Thalidomide Sodium arsenite (NaAs ₂ HO ₄) Diethylstilbestrol Tamoxifen Paclitaxel (Taxol) Phenytoin Rifampicin PCN AraC	Chemicals related to Chemical Substances Control Law in Japan	2,4-dinitrophenol 4-amino-2,6-dichlorophenol Pentachlorophenol 2-Aminomethylpyridine 2-Vinylpyridine 1,2,3-Triazole 1,2,4-Triazole 3-Amino-1H-1,2,4-triazole N-Methylaniline 2-Chloro-4,6-dimethylaniline 1,2-Dichloro-3-nitrobenzene 4-Ethynitrobenzene
Agricultural chemicals	Paraquat Methoprene Pyriproxyfen Tebufenozide Acephate Carbaryl Warfarin Permethrin Deet	Industrial chemicals	Toluene Bromobenzene Carbon tetrachloride Methanol DMSO Tributyltin Bisphenol A MEHP DEHP Fullerene Indigo
Food-derived chemicals	Citric acid Hydroxycitric Acid Forskolin Caffeine Monocrotaline Ethanol Coenzyme Q10 Genistein Genistin Daizein	DNA demethylating drugs inhalational toxic chemicals Agonists on nuclear receptor	Azacytidine Formaldehyde Acetaldehyde Ethynodiol Testosterone propionate Clofibrate Troglitazone Levothyroxine All trans retinoic acid 9-cis retinoic acid Methoprene acid TCDD TCDF 3-methylcholanthrene
Mutagen	Diethylnitrosamine N-ethyl-N-nitrosourea		





TCDD-inducible
poly(ADP-ribose)
polymerase





Thalidomide

Animal: C57BL/6 CrSlc, male, 12 weeks

Dose: 0, 80, 240, 800 mg/kg

Time: 2, 4, 8, 24 hr

p.o. (corn oil)

n=3

Organ: Liver

GeneChip (Affymetrix): MOE430 A = 22,690 probe sets (ps)

Normalization: PerceLlome

Data analysis

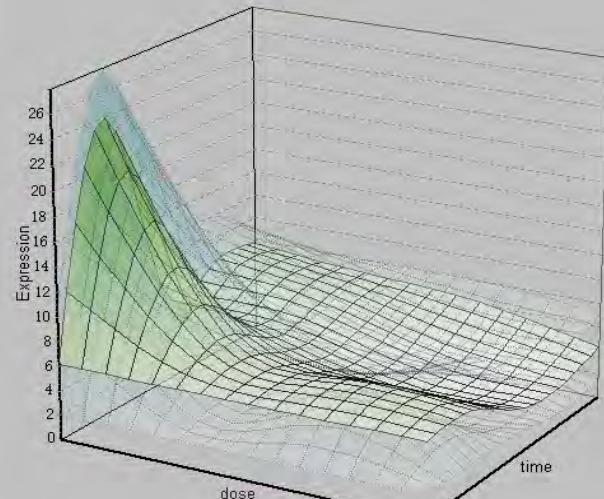
MF CMF: similarity

MF Surface: display expression pattern

Ddit4

1428306_at

1428306_at
Ddit4
AK017826

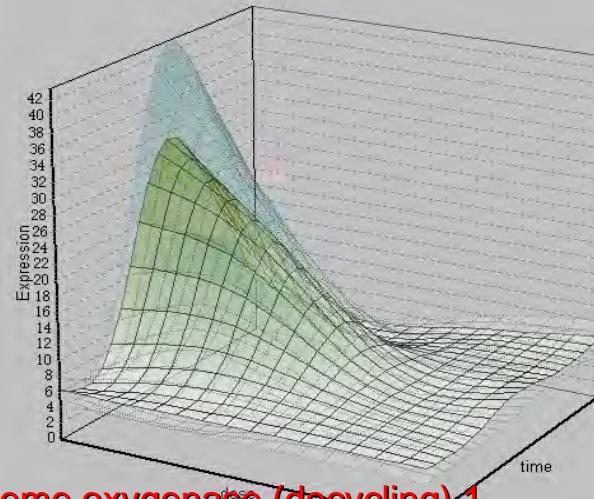


DNA-damage-inducible transcript 4

Hmox1

1448239_at

1448239_at
Hmox1
NM_010442

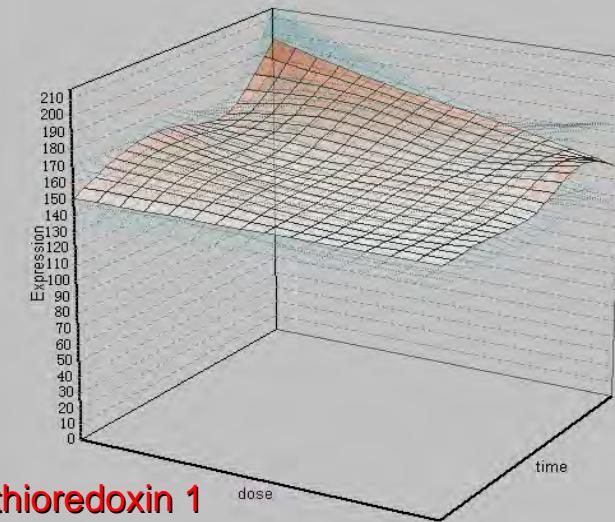


heme oxygenase (decycling) 1

Txn1

1416119_at

1416119_at
Txn1
NM_011660

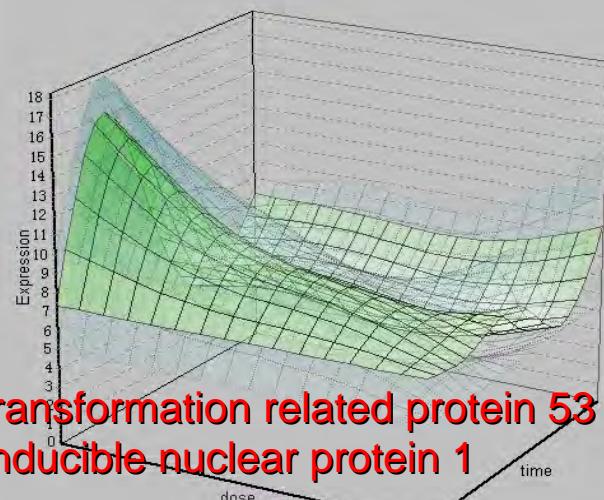


thioredoxin 1

Trp53inp1

1416927_at

1416927_at
Trp53inp1
AW495711

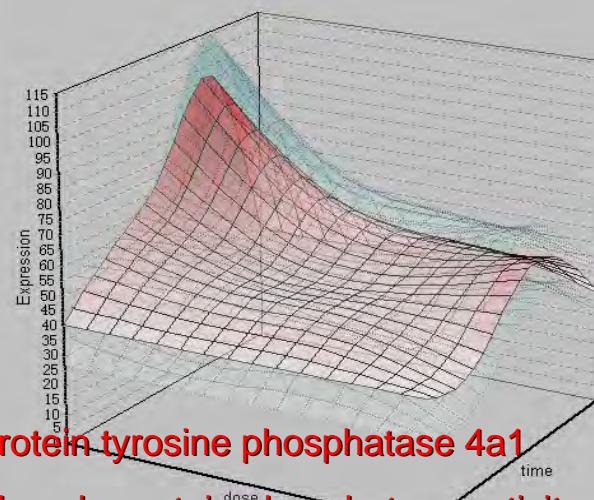


transformation related protein 53
inducible nuclear protein 1
apoptosis

Ptp4a1 /// LOC385423 /// LOC433406 /// LOC434095

1449322_at

1449322_at
Ptp4a1 /// LOC385423 /// LOC433406 /// LOC434095
BC003761

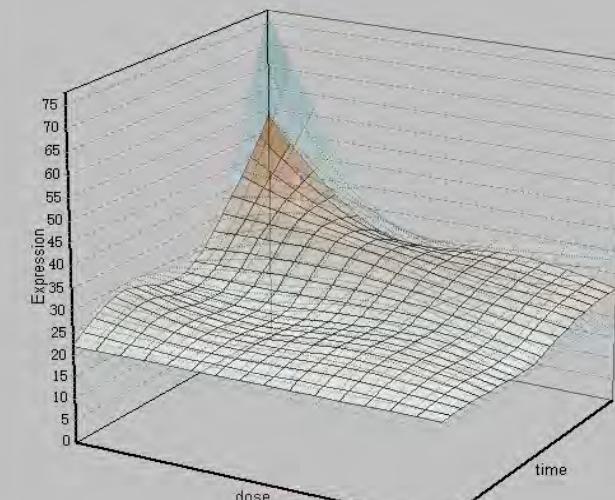


protein tyrosine phosphatase 4a1
phosphoprotein phosphatase activity

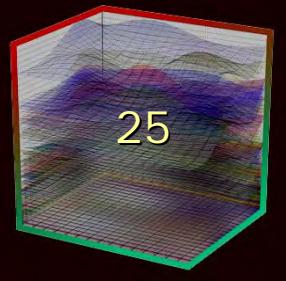
Cbr1

1460196_at

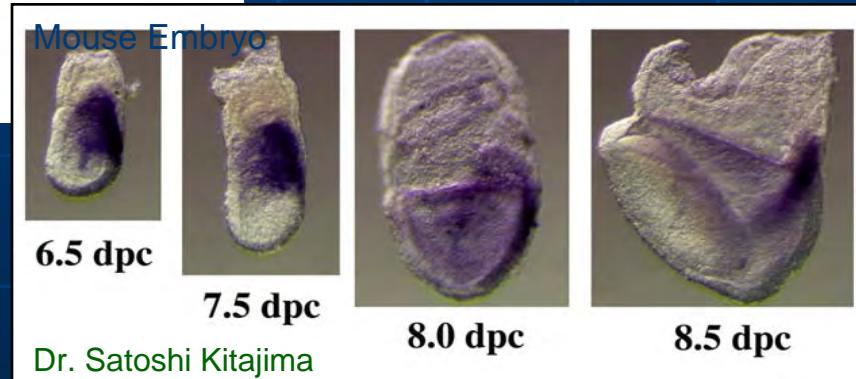
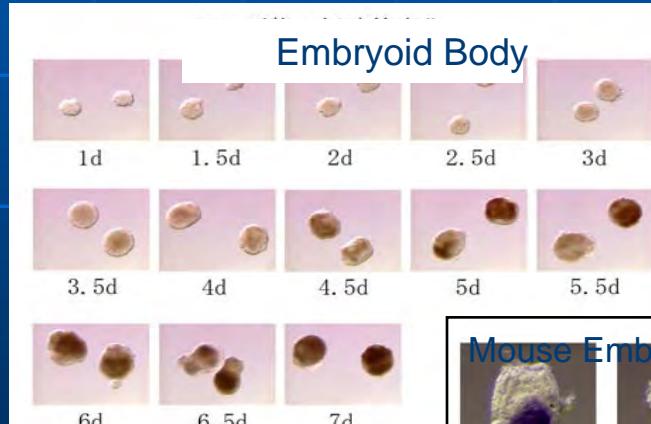
1460196_at
Cbr1
NM_007620



carbonyl reductase 1

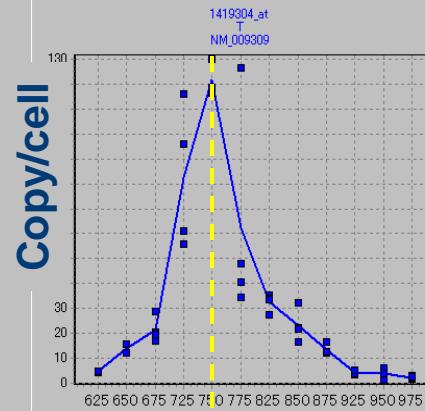


Fetus (Developmental) toxicogenomics (Percellome)

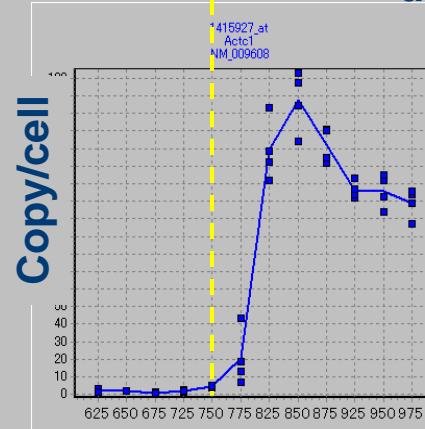


Brachyury

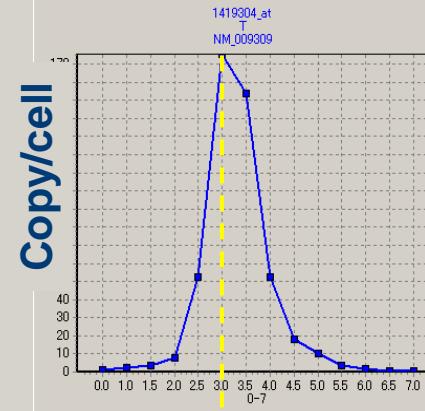
Whole Embryo



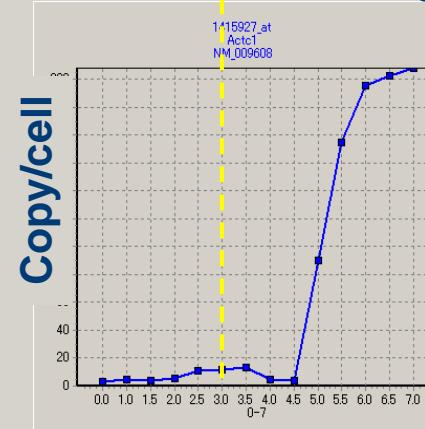
Cardiac actin



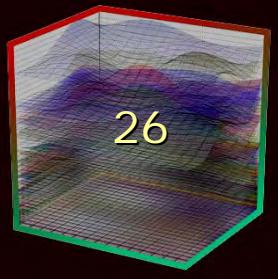
Embryoid Body



Brachyury

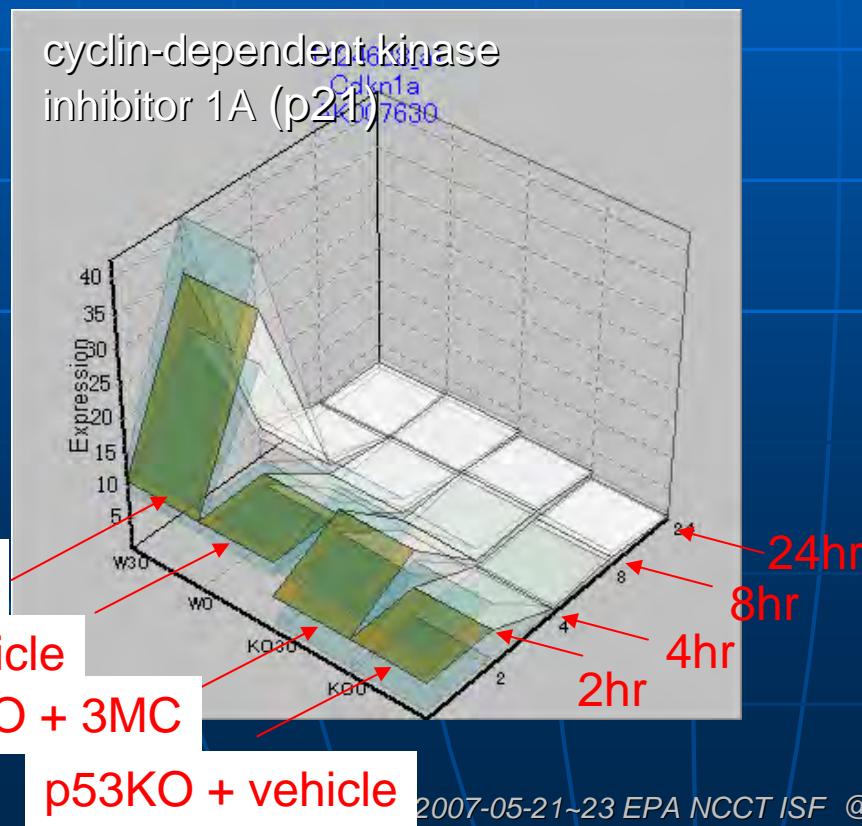


Cardiac actin



KO mouse study

p53 +/- mouse
wild type (C57BL/6)
3-methylcholanthrane (3-MC) 30gm/kg
Single gavage
time: 2, 4, 8, 24 hr (n=3)



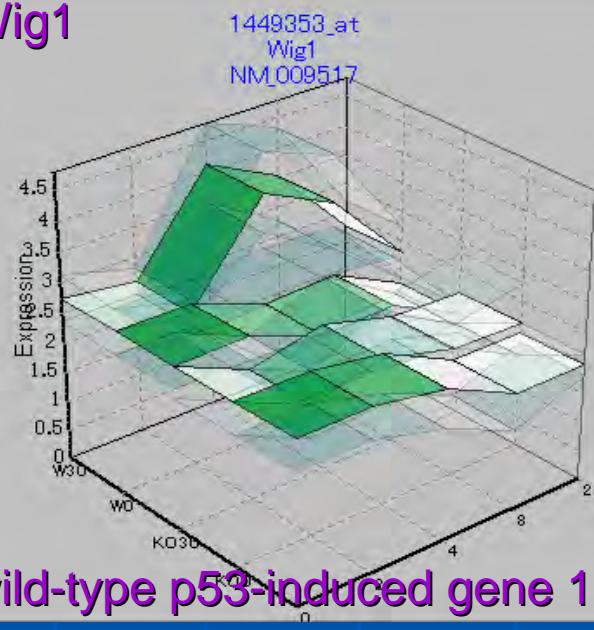
Search for p53-dependent genes

Search for p53-independent genes

Probe chemical = 3-MC
(AhR ligand as well as mutagen)

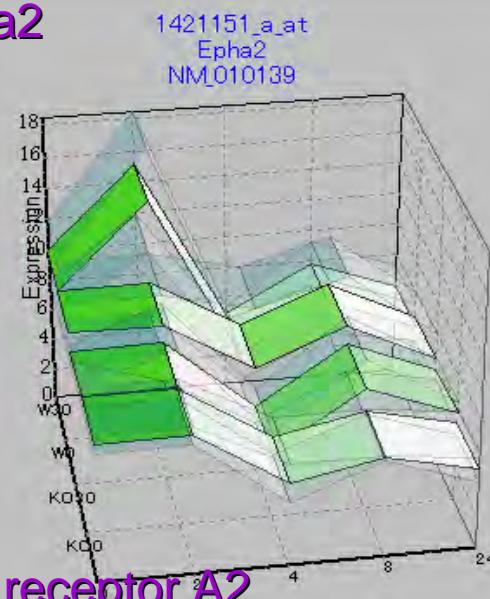
p21 (cdkn1a) is induced

Wig1



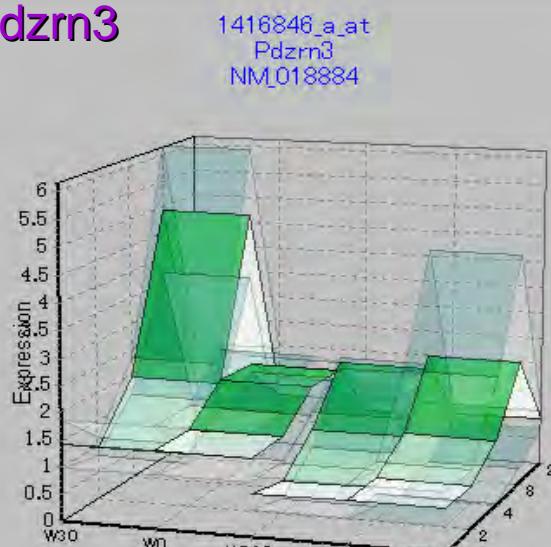
wild-type p53-induced gene 1

Epha2



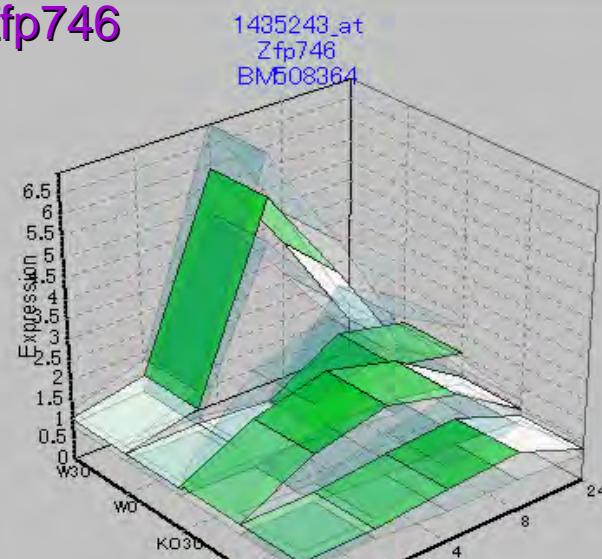
Eph receptor A2

Pdzrn3



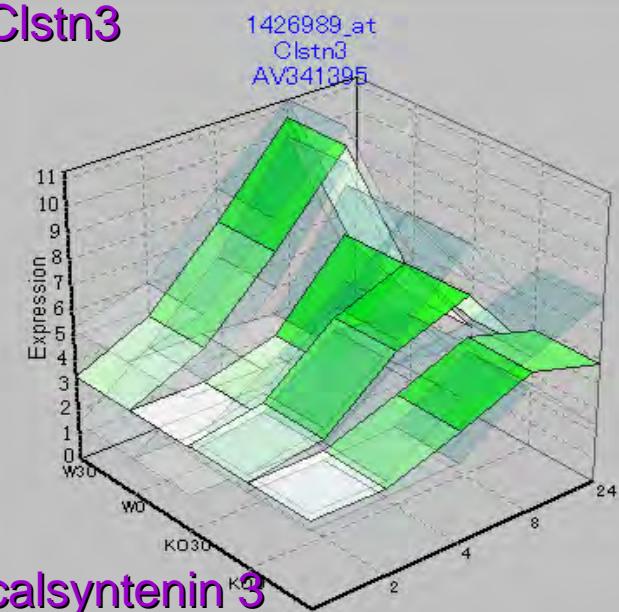
PDZ domain containing RING
finger 3

Zfp746

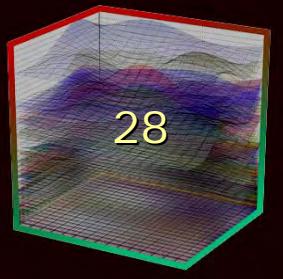


zinc finger protein 746

Clstn3



calsyntenin 3



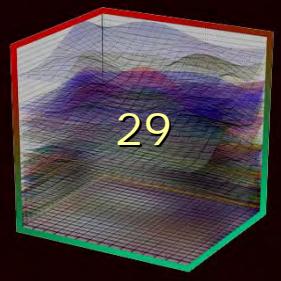
Multiorgan analysis

■ Pattern -1

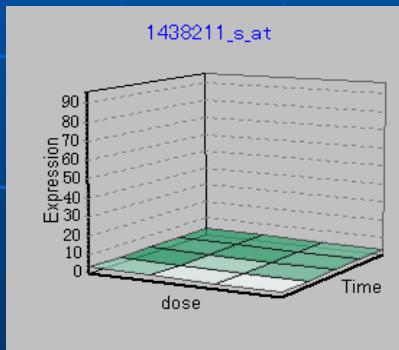
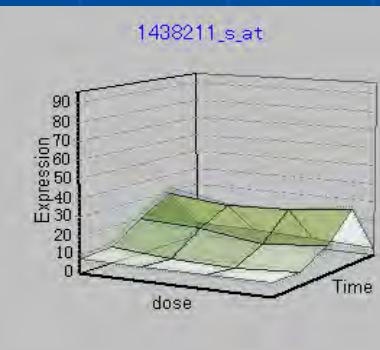
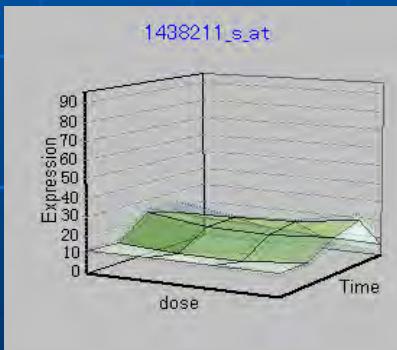
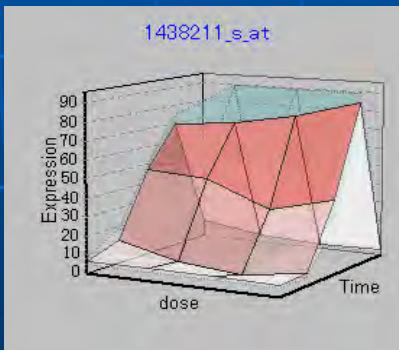
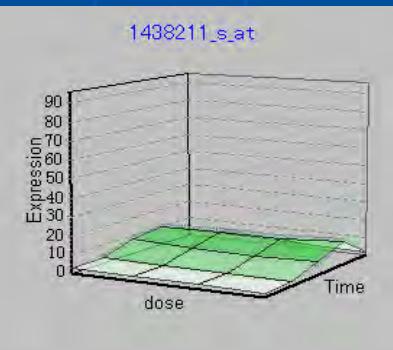
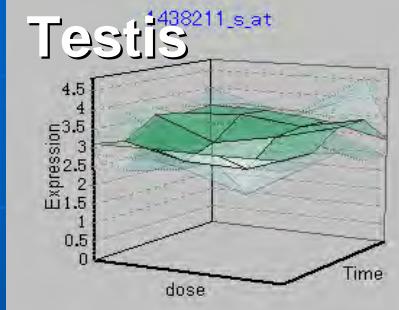
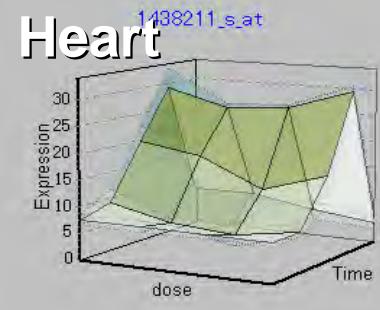
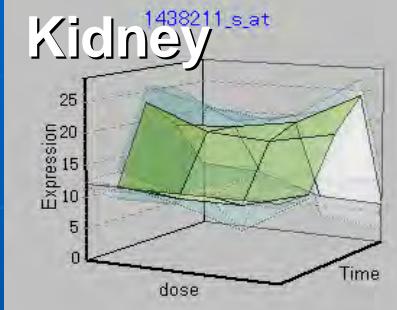
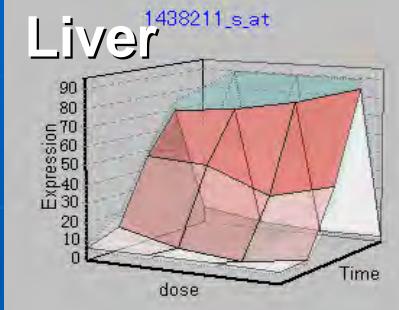
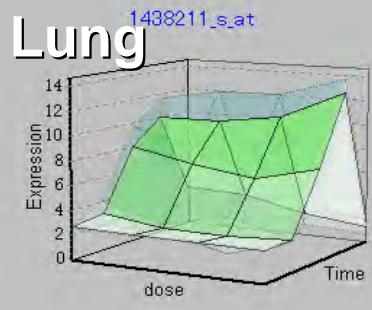
- Lung
- Liver
- Kidney
- Heart
- Testis

■ Pattern -2

- Brain
 - Cerebral cortex
 - Hippocampus
 - Brain stem
 - Cerebellum
- Liver

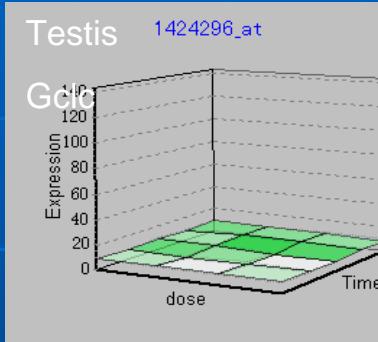
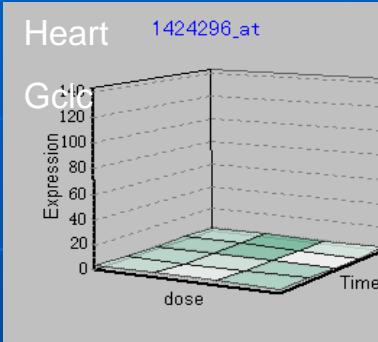
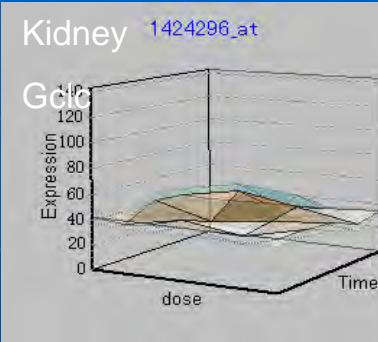
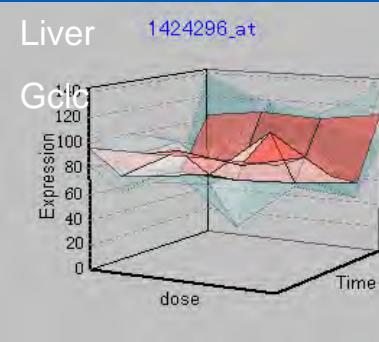
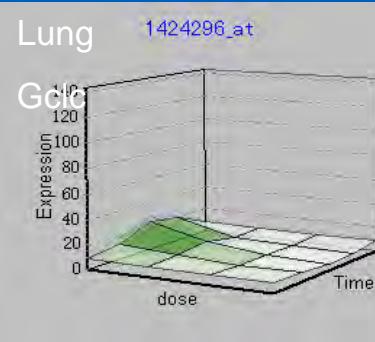
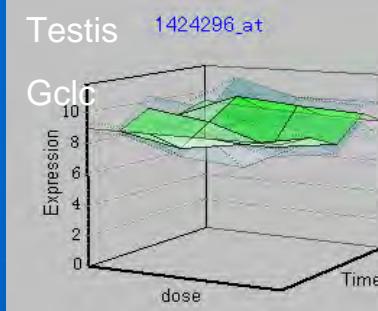
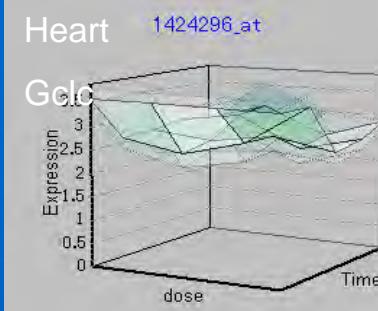
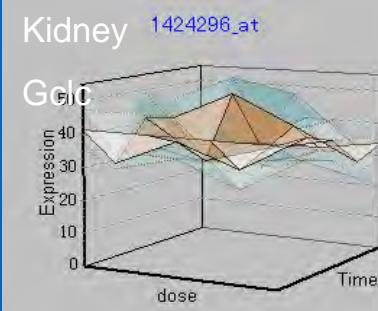
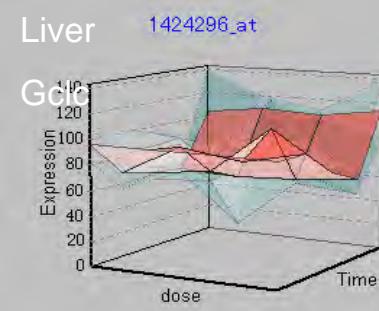
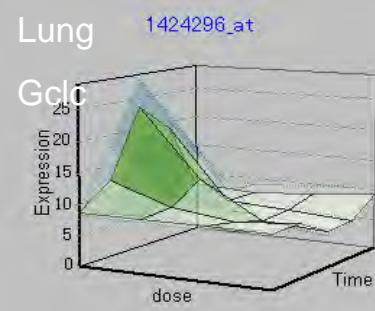


Circadian rhythm



Dbp

D site albumin promoter binding protein



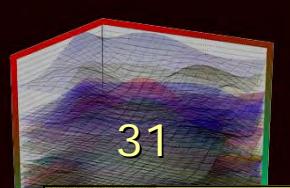
Pattern -1 CCl₄

Single oral gavage
2, 7, 20 mg/kg
time: 2, 4, 8, 24

Gclc

glutamate-cysteine ligase, catalytic subunit

Inhalation TG (Formaldehyde)



2hr single exposure

0.1, 0.3, 1.0 ppm

AM10:00
(hr) 0

24

48

72

96

120

144

168

exposure ↓ sampling

6hr repeated

0.03, 0.1, 0.45 ppm

AM10:00
(hr) 0

24

48

72

96

120

144

168

22hr repeated

0.03, 0.1, 0.3 ppm

PM0:00
(hr) 0

24

48

72

96

120

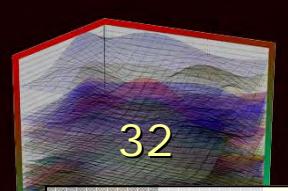
144

168

192

light cycle

PM8:00 AM8:00



Inhalation TG (Formaldehyde)

2hr single exposure

AM10:00 暴露開始
(hr) 0 24 48 72 96 120 144 168 192

6hr repeated

AM10:00 暴露開始
(hr) 0 24 48 72 96 120 144 168

22hr repeated (7d)

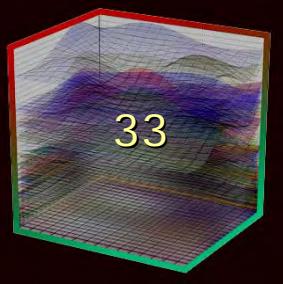
PM0:00 暴露開始
(hr) 0 24 48 72 96 120 144 168 192

PM8:00 AM8:00

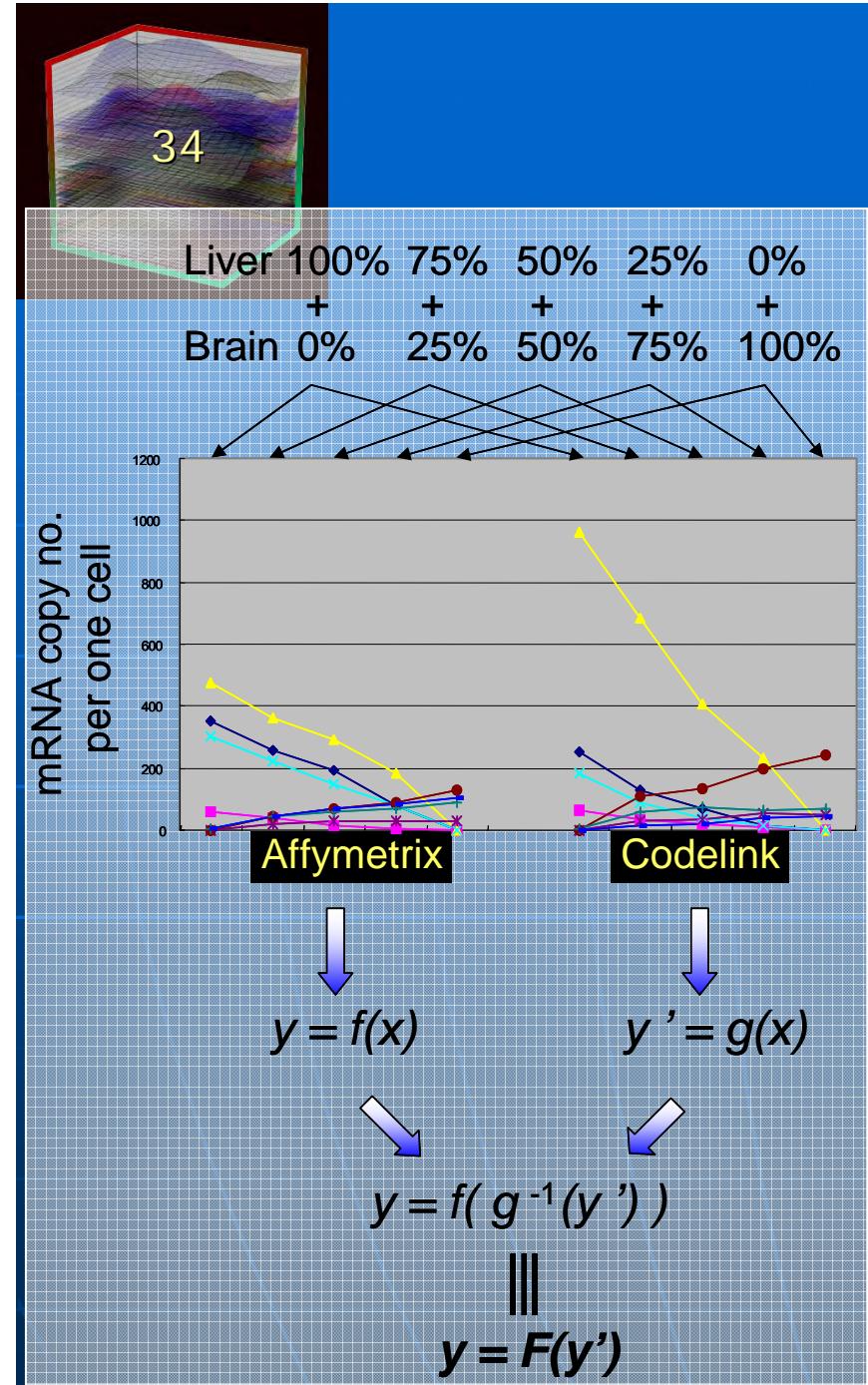
2007-05-21~23 EPA NCCT ISF @ RTP, NC

曝
露

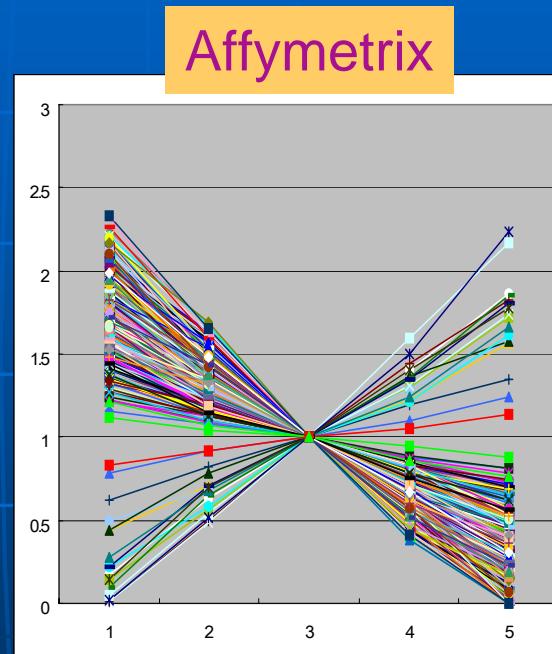
解剖



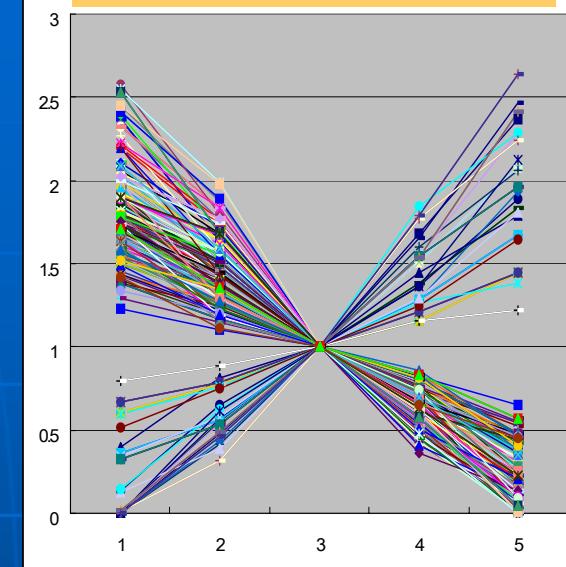
Data-bridging among Affy, Agilent and Q-PCR



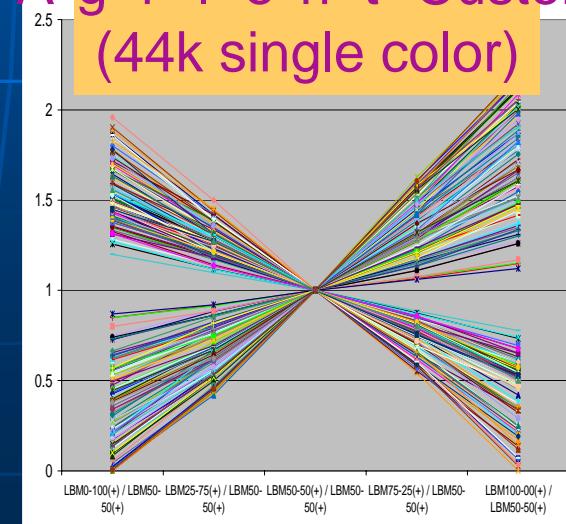
To 250
High linearity
probesets
(R^2 closest to 1)

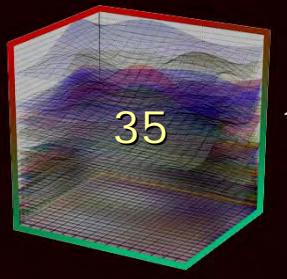


CodeLink Custom



Agilent Custom
(44k single color)

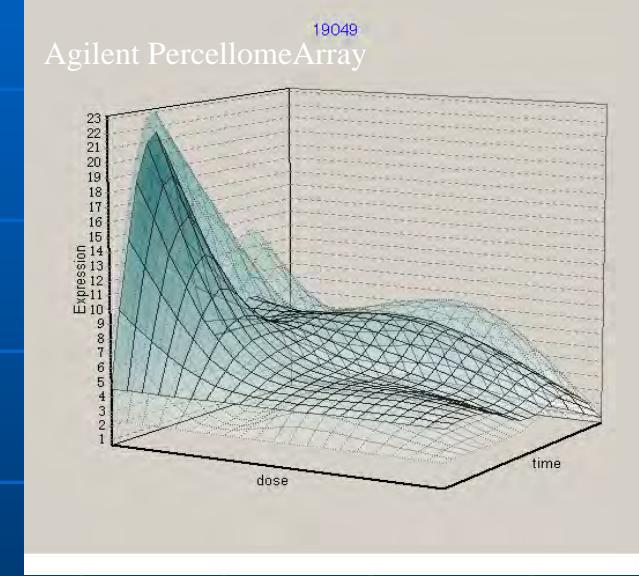
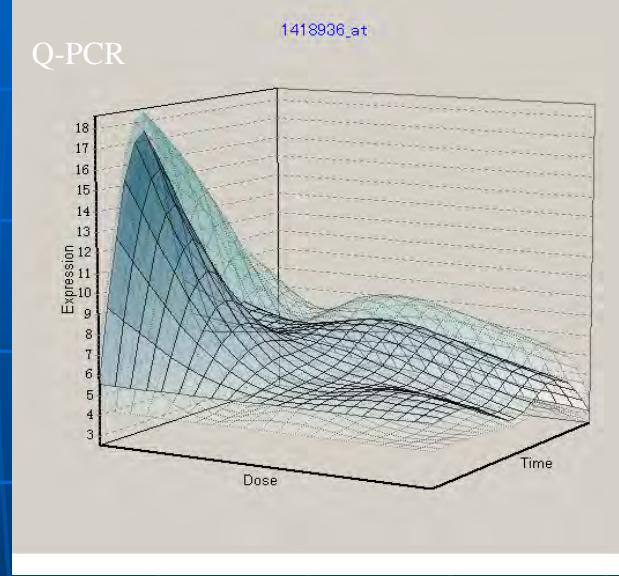
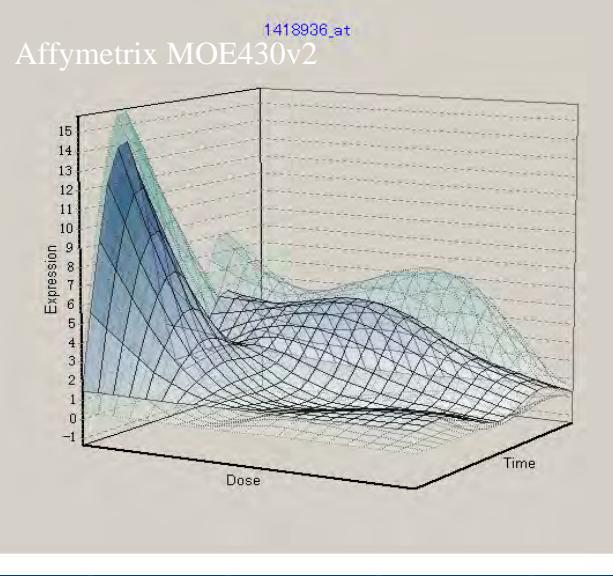




Affy

Q-PCR

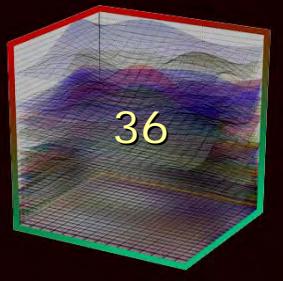
Agilent



- A2A_ByUnigene@
 - MOE430v2
 - 1418936_at
 - AgilentID
 - A_52_P608322
 - AgilentNIHS44K
 - 19049

Maff

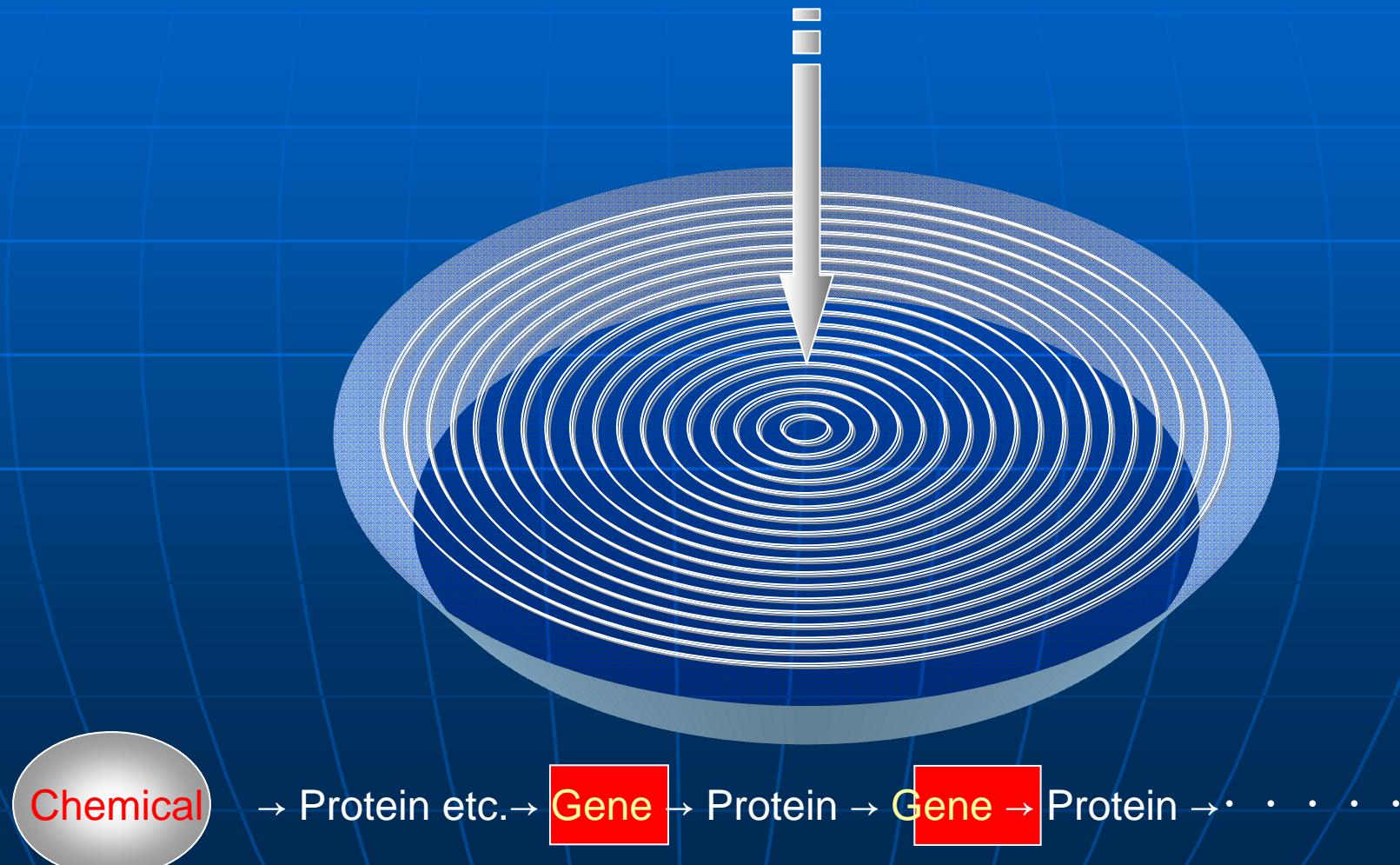
2007-05-21~23 EPA NCCT ISF @ RTP, NC

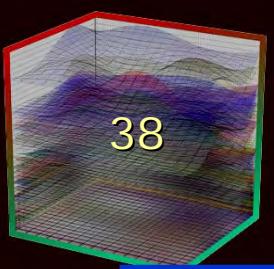


Strategy of data analysis (single gavage studies for early responses)

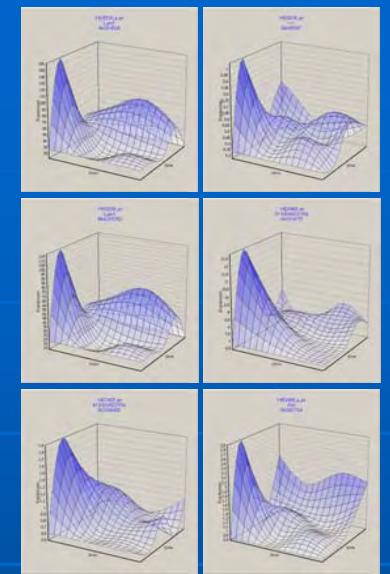
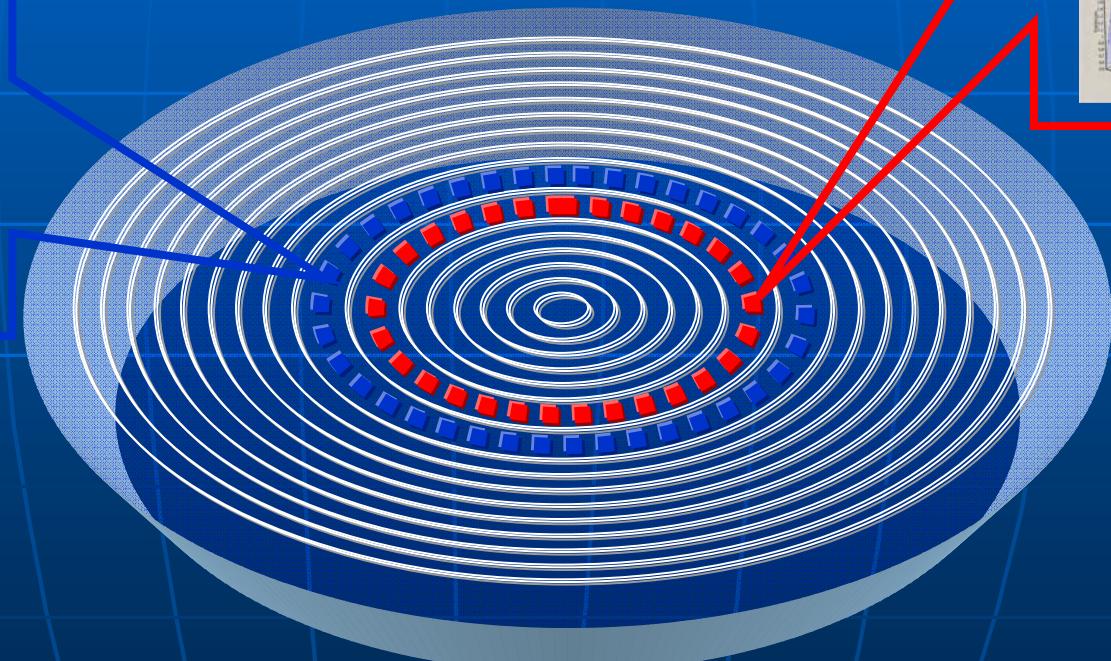
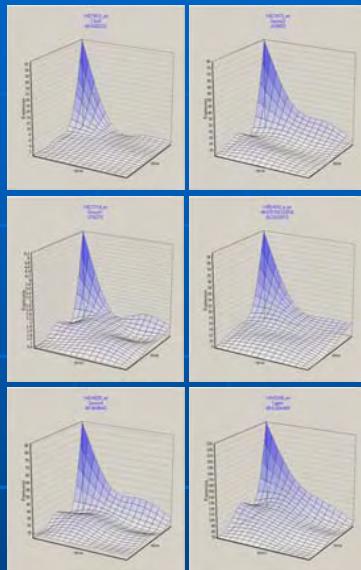
Single exposure experiment

- Gene Cascade = water surface
- Test Chemical = stone
- Alteration in gene expression = ripple





38



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'Millefeuille(MF)' analysis system:

39

expression pattern-based
**automatic candidate gene
extraction**

isoborograms of all genes

mRNA copy no. per one cell

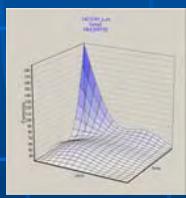
unsupervised clustering

(Mass Distributed Clustering*)

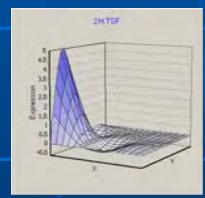
- extracting unique expression-patterned genes
- sort 50,000 genes into 1,000 clusters

*collaboration with
NTT COMWARE and
Teradata (NCR)

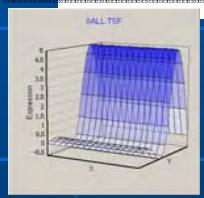
focused gene
(24hr peak)



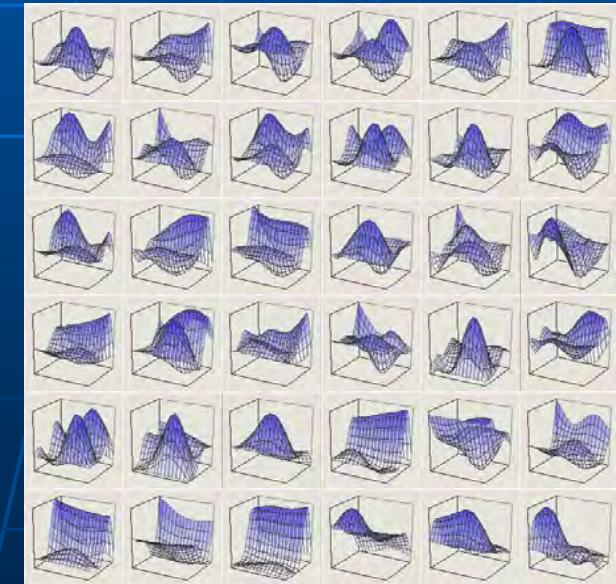
virtual pattern
(early response)



virtual pattern
(circadian type)



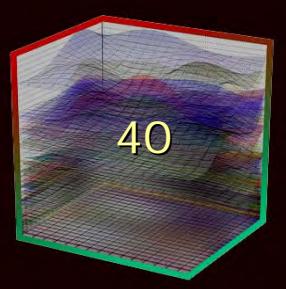
central pattern of each cluster



S. Matsumoto, et al.

Mass Distributed
Clustering: A New
Algorithm for Repeated
Measurements in
Gene Expression Data.

Genome Informatics
(2005), 16, 183-194



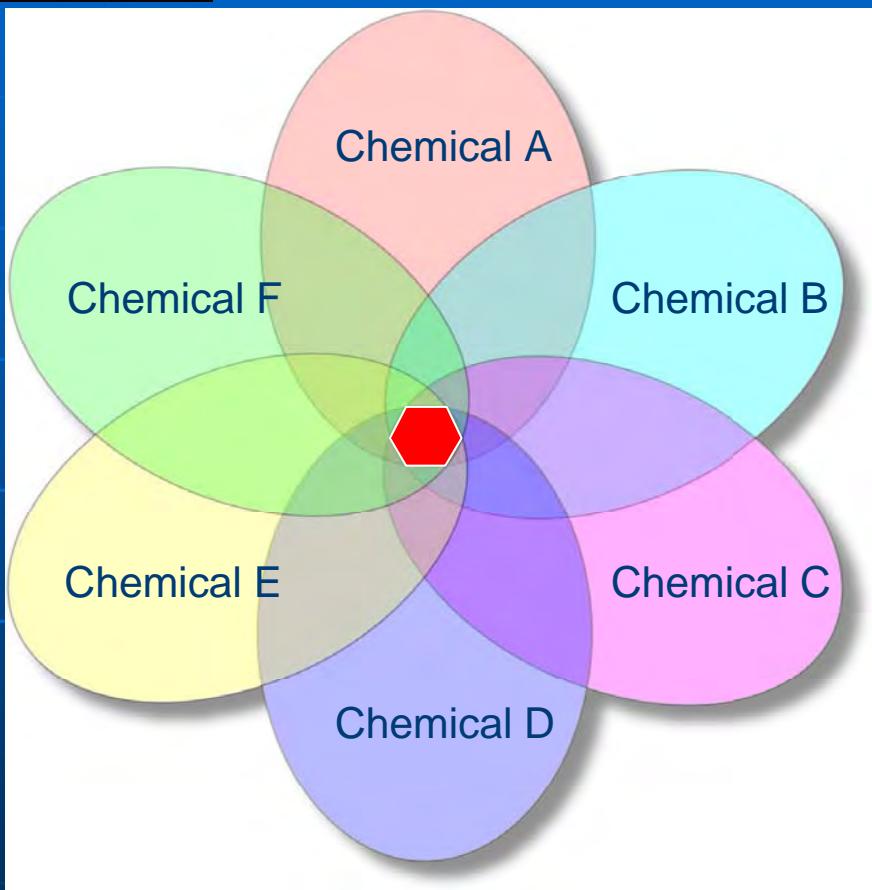
Chemical A

Chemical B

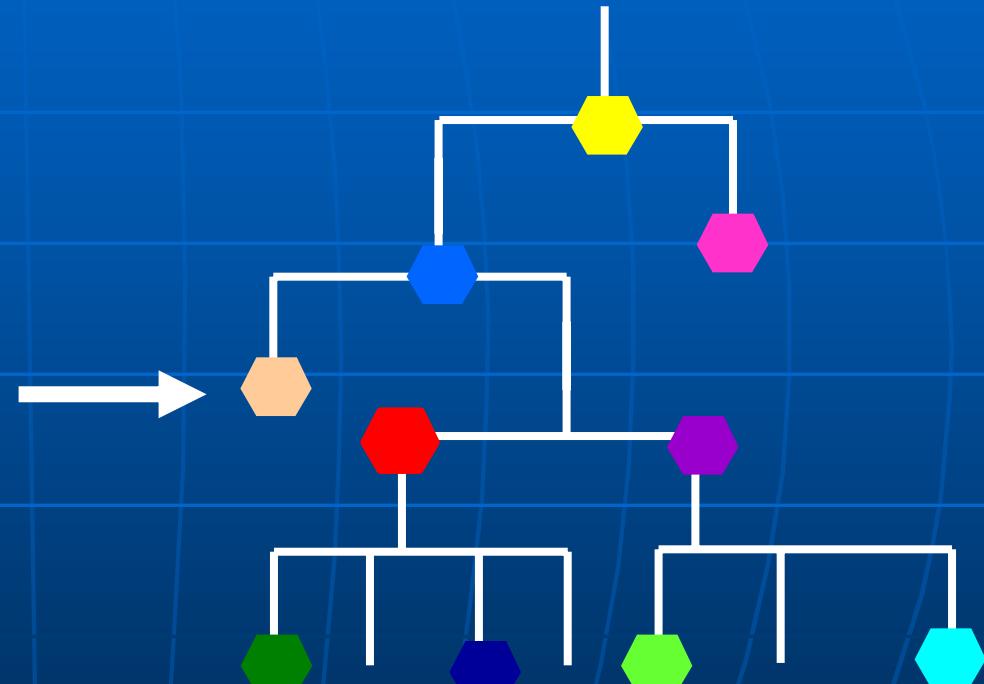
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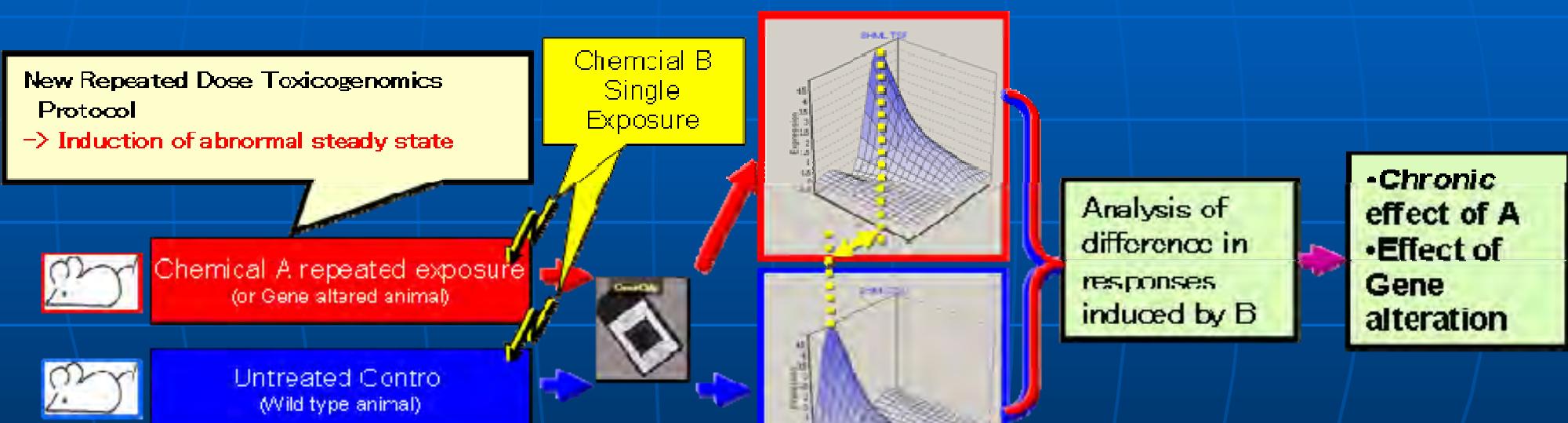
“synchronized” gene concept

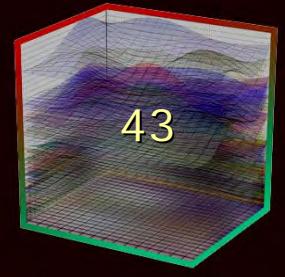


Signal cascade



New concept of Repeated dose Toxicogenomics (which should enable direct comparison with Gene KO mouse data)

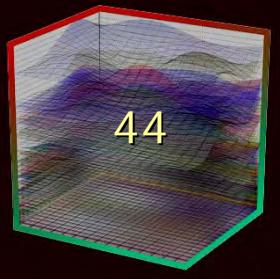




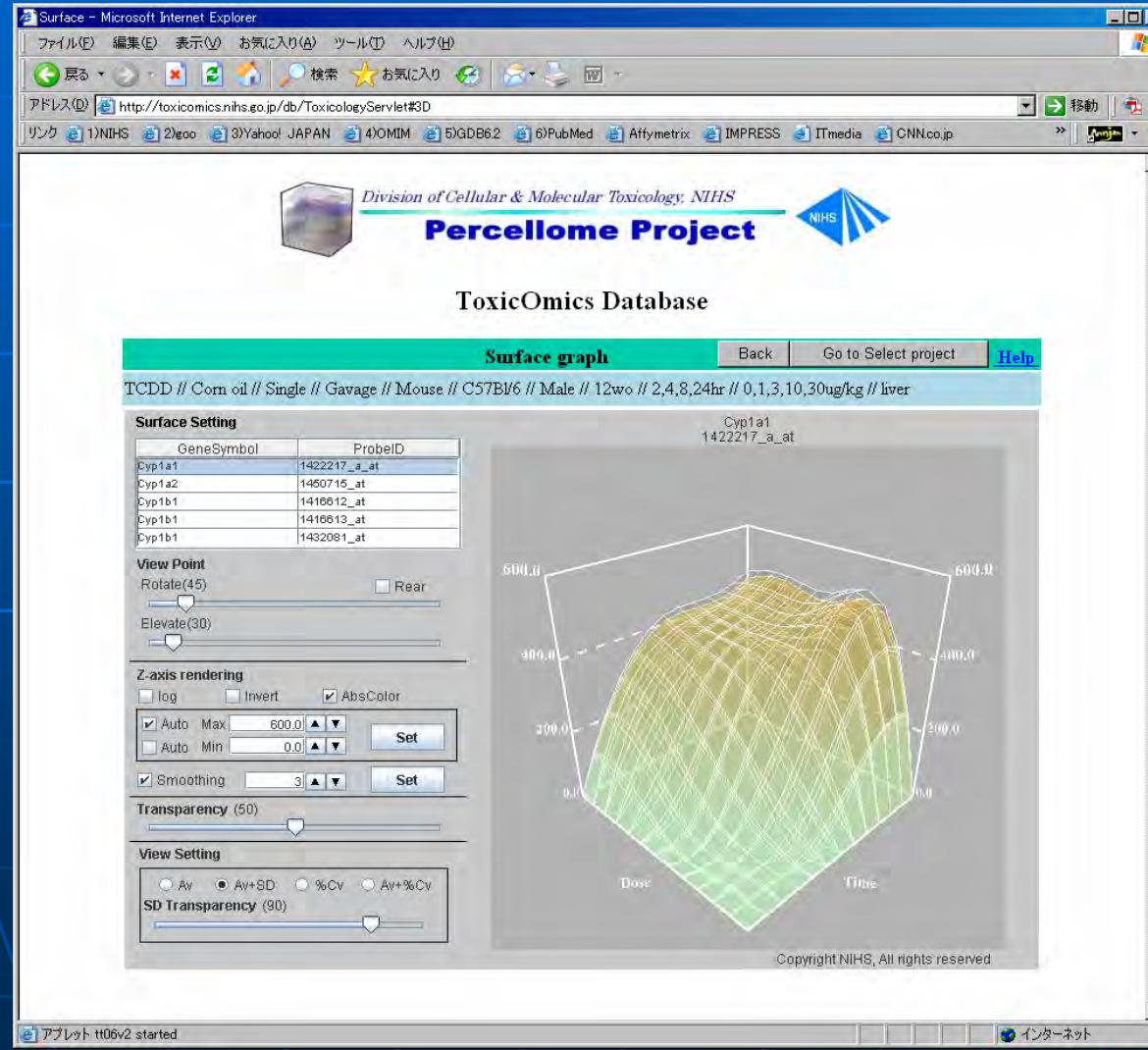
43

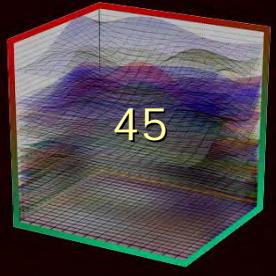
Summary

- Percellome method is developed for easy and direct comparison among samples/studies (and platforms).
- Percellome Toxicogenomics database (mouse liver, single gavage, early responses at 2, 4, 8, and 24 hrs) is developed (90~chemicals).
- Repeated dose data by new chronic study concept will be generated in next few years.
- Fetus Percellome approach seems to be very promising for developmental toxicity studies.
- Inhalation toxicogenomics provides with higher sensitivity at low concentration near the “Sick building syndrome” levels.



<http://toxicomics.nihs.go.jp/db/>

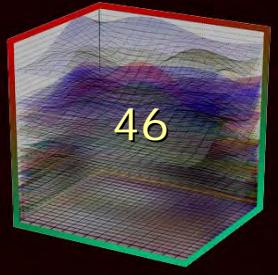




Percellome essentials

- dose-graded spike cocktail; GSC
 - synthesized from ATCC clones
- DNA quantification protocols (manual and for PerkinElmer JANUS robot)

GSC stock and protocol are available on a collaboration basis, please contact kanno@nihs.go.jp.



Development of Percellome (2001~)

Jun Kanno, MD, PhD
Katsuhide Igarashi, PhD
Ken-ichi Aisaki, MD, PhD
Atsushi Ono, PhD
Tomoko Ando, Ms
Noriko Moriyama, Ms
Yuko Kondo, Ms
Yuko Nakamura, Ms
Maki Abe, Ms

NIHS TGP (with 17 Pharm) startup group (~ summer 2002)

Akihiko Hirose	Risk Assess/ BSRC/ NIHS
Takayoshi Suzuki	Mutagen/ BSRC/ NIHS
Makoto Shibutani	Path/ BSRC/ NIHS
Katsuhide Igarashi	Tox/BSRC/NIHS
Atsushi Ono	Tox/BSRC/NIHS
Ken-ichi Aisaki	Tox/BSRC/NIHS
Jun Kanno Tox/BSRC/NIHS	

Grants

Ministry of Health, Labor, and Welfare (MHLW) Grant-in-Aid H18-Kagaku-Ippan-001, H15-kagaku-002, H14-Toxico-001, H13-seikatsu-012, & MOE

Percellome Projects (2003~)

Ken-ichi Aisaki, MD, PhD
Katsuhide Igarashi, PhD
Noriyuki Nakatsu, PhD
Yukio Kodama, DVM
Tomoko Ando, Ms
Noriko Moriyama, Ms
Yuko Kondo, Ms
Yuko Nakamura, Ms
Maki Abe, Ms
Kenta Yoshiki, Mr
Nae Matsuda, DVM
Chiyuri Aoyagi, Ms
Kouichi Morita, Mr
Ayako Imai, Ms
Shinobu Watanabe, Ms
Yukio Ogawa, DVM (Inhalation)
Satoshi Kitajima DVM, PhD (Fetus)
Kentaro Tanemura PhD

Millefeuille Softwares

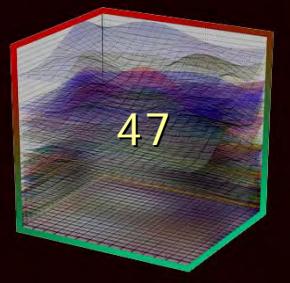
Ken-ichi Aisaki, MD, PhD

IT collaboration

NTT COMWARE
with Teradata, NCR
(Shinya Matsumoto,
Bun-ichi Tajima)

Percellome Collaborator Scientists

Dr. Shigeaki Kato
Dr. Yoshiaki Fujii-Kuriyama
Dr. Bruce Blumberg
Dr. Hironobu Sasano
Dr. Yumiko Saga
Dr. Seiichi Hashimoto
Dr. Yasufumi Shigeyoshi
and others



■ END